

# ABSTRACT BOOK

European Association for Vision and Eye Research  
 EVER  
European Association for Vision and Eye Research  
[www.ever.be](http://www.ever.be)

EVER 2013  
September 18-21  
Science for Sight  
NICE

E V E R



European Association for Vision and Eye Research

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## EVER 2013 Lecture: Advances in OCT imaging

*Leopold SCHMETTERER*

*Clinical Pharmacology (Vienna)*

*Medical Physics and Biomedical Engineering (Vienna)*

### **Summary**

In the brain there is a tight coupling between local neural activity and local blood flow and metabolism. This phenomenon called neurovascular coupling was proposed more than 100 years ago and since then confirmed by numerous experiments. A breakdown in functional hyperemia has been hypothesized to contribute to loss of neurons in diseases such as stroke, hypertension, spinal-cord injury and Alzheimer's disease. In the eye this phenomenon exists as well. During stimulation of the eye with flicker light there is a pronounced increase in retinal and optic nerve head blood flow. The mechanisms underlying neuromuscular coupling in the retina are complex and involve the interaction between neurons, astrocytes and blood vessels. In this process vasoactive arachidonic acid metabolites play a key role. In addition, the vascular endothelium is involved. The response of retinal vessels to flicker stimulation is also modified by nitric oxide and oxygen. Neurovascular coupling is altered in diseases such as glaucoma or diabetes and may contribute to neuronal loss in these diseases. A better understanding of these phenomena is required to achieve neuroprotection via the neuromuscular pathway.



## Major Central European's contributions to international ophthalmology

Andrzej GRZYBOWSKI

*Department of Ophthalmology, City Hospital (Poznan)*

*DDepartment of Ophthalmology, University of Warmia and Mazury (Olsztyn)*

### Summary

Major Central European's contributions to international ophthalmology

The fundamentals of modern ophthalmology were created in Europe in 18th and 19th centuries. At that time, Central European states were not independent, and majority were occupied by neighbouring countries, like Russia, Prussia and Austro-Hungary. This political situation significantly limited the development of science and medicine in those countries. Scientific development was hindered by such factors as poor investment in research, decreased number of universities, emigration of some researchers due to political reasons or due to the lack of adequate academic background. In spite of numerous complications, many interesting discoveries and observations were made in Central Europe at that time, even though the origin of most of them is now forgotten. The aim of this study is to present some major achievements of important contributors to international ophthalmology originating from Central Europe, including Witelo, Purkinje, Szokalski, Wicherkiewicz, Galezowski, Borysieckiewicz, Goldzieher, Blaskovics, Imre Jr., Kettesy, Krwawicz, Wilczek, Wichterle and others.



## Acta Ophthalmologica

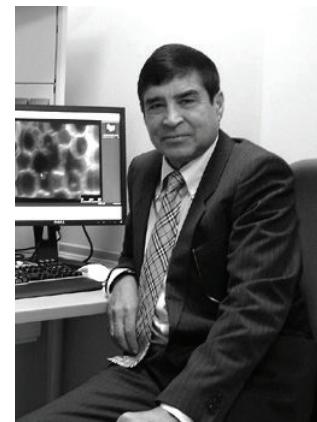
### Telomere maintenance and retinal vascularisation

*Tero KIVELÄ  
Helsinki*

#### Summary

Abnormal development of the retinal vasculature is an important cause of vision loss in infancy, childhood and, occasionally, in adult life. In addition to retinopathy of prematurity from exposure to oxygen after preterm birth, several genetic defects affecting retinal vascularization have recently been discovered, affecting either Wnt signaling or telomere maintenance. The canonical norrin–beta-catenin pathway of Wnt signaling is deranged by mutations in frizzled-4 (FZD4), low density lipoprotein receptor-related protein 5 (LRP5), norrin (NDP) and other genes. These lead to vascular leakage, vitreoretinal traction or both in Coats' disease, familial exudative vitreoretinopathy (FEVR), Norrie's disease and osteoporosis-pseudoglioma syndrome. Mutations in telomeric repeat-binding factor 1-interacting nuclear factor 2 (TINF2) and CTS telomere maintenance complex component 1 (CTC1) in Revesz syndrome and cerebroretinal microangiopathy with calcifications and cysts (CRMCC) can even cause retinal angiomas. Wnt signaling initially was the suspect for them as well, because all six diseases share more or less widespread retinal avascularity as a hallmark. Indeed, a link between the two pathways is still possible.

The manifestations of the telomere-related syndromes are more protean than those of Wnt-related ones, which have no other significant systemic findings than osteoporosis if LRP5 is mutated. The former may lead to a cerebral and intestinal vasculopathy or hepatopathy, all of which can be fatal, and to skin and hair abnormalities. Milder forms can escape diagnosis until adulthood. It is useful to recognize these genetic vasculopathies. Genes for cases which still go unexplained remain to be discovered.



### The neuroscience of glaucoma in relation to the possibility for neuroprotection

Neville OSBORNE  
Fundación de Investigación Oftalmológica (Oviedo)  
Nuffield Dept.Clinical Neurosciences (Oxford)

#### Summary

Various theories exist to explain how glaucoma is initiated to result in the differential rate of retinal ganglion cell death. We suggest that initially the quality of the blood supply in the optic nerve head region is affected to cause a type of ischemia. This causes an alteration in ganglion cell mitochondrial homeostasis and an activation of astrocytes and other glia in the optic nerve head. Thereafter, as the disease progresses substances released from activated glial cells and also blue light reaching the retina act synergistically to cause the death of specific ganglion cells at different times. We therefore propose that the repertoire of receptors and number of mitochondria in individual ganglion cells relate to their time of death after glaucoma is initiated. These ideas will be presented, as they suggest that the causes and mechanisms for individual ganglion cells dying in glaucoma vary. Such a theory implies that substances with a single mode of action is unlikely to be sufficient for effective clinical neuroprotection but that this might be achievable using substances with multiple modes of action or a suitable cocktail mixture of products.



## Why what you have been taught about the optic disc may not be entirely true

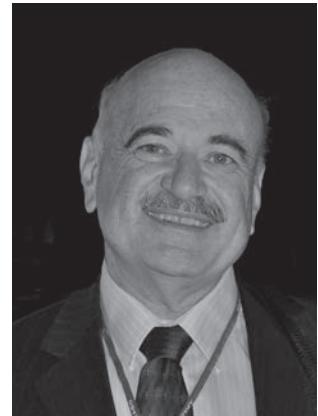
*Balwantray CHAUHAN  
Dalhousie University (Halifax)*

### Summary

Clinicians evaluate neuroretinal rim health according to the appearance of the optic disc, the clinically visible surface of the optic nerve head (ONH). Recent anatomic findings with optical coherence tomography challenge the basis and accuracy of current rim evaluation for 3 reasons:

- (1) The DM is rarely a single anatomical structure or an identifiable junction, such as the inner edge of border tissue. In most eyes it corresponds variably to multiple anatomical structures.
- (2) In some regions of all ONHs, Bruch's membrane extends internally beyond the DM (towards the centre of the ONH) and is both clinically and photographically invisible. Since in these areas the outer border of the rim is the termination of Bruch's membrane and not the more external DM, the rim is narrower than that with clinical or photographic evaluation.
- (3) Because current rim width measurements are made in a fixed plane without reference to the orientation of the rim tissue, for the same number of axons, the rim width will be greater in cases where the orientation of the rim tissue is more horizontal (for example in the temporal sector of tilted optic discs) compared to when it is more perpendicular.

This presentation will review and interpret ONH anatomy detected with optical coherence tomography pertaining to optic disc examination and demonstrate why a paradigm change for clinical assessment of the optic nerve head is now necessary.



## Why is the optic nerve the canary in the coal mine of mitochondrial diseases?

Alfredo SADUN

Ophthalmology and Neurosurgery at USC Sch. Med. (Los Angeles)  
Doheny Eye Institute (Los Angeles)

### Summary

Mitochondrial Optic Neuropathies (MON) present characteristic clinical and pathological features. Loss of visual acuity, dyschromatopsia and a central scotoma speak to involvement of the papillomacular bundle (PMB). Histopathology confirms this. These features are seen in hereditary diseases (Leber's Hereditary Optic Neuropathy—LHON and Dominant Optic Atrophy), Syndromes (such as MELAS, Wolfram's or FA), or toxic MONs. So why is the optic nerve involved so early and so often in mitochondrial disease? The brain weighs 2% of the body but consumes 20% of the body's oxygen. This expensive consumption of energy is largely due to the need for repolarization of the axon's membrane after each action potential. This is largely mitigated by myelin such that the sodium/potassium pumps only work at the Nodes of Ranvier. However, the retinal nerve fiber layer remains unmyelinated due to the need for inner retinal transparency. We present a mathematical model of these conditions that predicts the order of fiber loss in MON as a function of myelin and axon diameter. We demonstrate, with morphometry of postmortem samples of normal and LHON optic nerves, that this pattern is strictly observed.



## Molecular sensors for the decoding of homeostasis disruptions in the retinal pigment epithelium: towards the understanding of retinal degenerative diseases

*Nicholas BAZAN*

*Neuroscience Center of Excellence and Department of Ophthalmology, School of Medicine, Louisiana State University Health Sciences Center (New Orleans)*

### **Summary**

The significance of the selective enrichment in omega-3 essential fatty acids DHA, docosahexaenoic acid) in photoreceptor cells has remained, until recently, incompletely understood. We contributed to the discovery of a docosanoid synthesized from DHA by 15-lipoxygenase-1, which we dubbed neuroprotectin D1 (NPD1: 10R,17S-dihydroxy-docosa-4Z,7Z,11E,13E,15E,19Z hexaenoic acid). NPD1 is promptly made on demand when homeostasis is at stake, as in oxidative stress ,proteostasis dysfunctions and in early stages of neural injury, ischemia-reperfusion or neurodegenerations. Thus NPD1 is a protective sentinel, one of the very first defenses activated when cell homeostasis is threatened . The availability of anti-apoptotic BCL-2 proteins is positively modulated by NPD1, whereas pro-apoptotic BCL-2 proteins are negatively regulated, as is activated microglia. Neurodegenerative diseases, in addition to enhancing oxidative stress, disrupt the proteostasis network and leads to a cellular inability to scavenge structurally damaged abnormal proteins.

The RPE cell response cascade potentiates disruptors of homeostasis through multiple checkpoints and signaling networks. NPD1 , a key component of this response and of the lipidomic signature, targets neuroinflammatory signaling and proteostasis and in turn promotes homeostatic regulation of the transcription of key genes that in turn act as molecular decoders of input, and thus results in cell survival.

(Supported by NIH: NINDS R01 NS046741, NEI R01 EY005121)

**WEDNESDAY  
SEPTEMBER 18  
2013**

## Course 1: Macular hole

### • 1411

#### **Pathophysiology of macular hole and diagnostic tools**

STAPPLER T

*Royal Liverpool University Hospital, Liverpool*

Purpose Our understanding of the pathophysiology of macular hole development has evolved as the diagnostic tools at our disposal have become increasingly sophisticated. Methods Critical review of current published literature regarding pathogenesis of macular hole formation and an appraisal of modern imaging tools such as SD-OCT, SLO-microperimetry in diagnosis and prognostication. Results Perifoveal PVD with vitreomacular adhesion and vitreoschisis allow cortical vitreous to remain on the retinal surface and act as a scaffold for cell proliferation. The presence of a contiguous photoreceptor inner/outer segment (IS/OS) line indicating well-restored photoreceptor cells is increasingly becoming a measure of functional success. Combined SD-OCT and SLO microperimetry studies suspect that ILM peeling may reduce retinal sensitivity. Conclusions Macular hole size seems to be among the best prognostic factors available, however several computerised algorithms as well as the presence or absence of an intact IS/OS layer may correlate with visual outcome. Changes in retinal morphology such as the length of the foveal cone outer segment tips line defect may prognosticate visual acuity after macular hole closure.

### • 1412

#### **ILM peeling or not - The best dye**

POURNARASJA

*Jules Gonin Eye Hospital, Lausanne*

The decision to peel or not ILM remains major controversy in macular hole surgery. A critical review of advantages and inconveniences will be discussed, as ILM peeling has been suggested to reduce retinal sensitivity when performed during surgery. Furthermore, we will review the different available dyes in order to peel the ILM in safe conditions.

### • 1413

#### **Posture face-down or not? The best tamponade**

FRIMPONG-ANSAH K

*Plymouth*

Post-operative posturing is an arduous and inconvenient process not without adverse effects. The purpose of this presentation is to assess whether or not carrying out face-down post operative posturing after macular hole surgery provides any benefit to the patient. A review has been carried out of current literature including randomised controlled trials and Cochrane Review related to posturing / positioning following macular hole surgery. The results of this review shows that there is no single clear conclusive approach. There may however be some situations, such as macular hole size which may have a bearing on the choice of whether or not to posture. Future randomised controlled trials may shed more light on this area of ophthalmic management.

### • 1414

#### **20g, 23g, 25g or 27g - The best forceps**

GOTZARIDIS S

*Athens*

#### **ABSTRACT NOT PROVIDED**

## Course 1: Macular hole

### • 1415

#### Partial thickness macular holes and pharmacological treatment

GEORGALAS I

*Department of Ophthalmology, Athens University, Athens*

Partial thickness macular holes and lamellar macular holes have a different pathophysiological mechanism compare to the full thickness macular holes as well as different management. Surgery is not always an option. This is the case for a group of full thickness macular holes as well, where pharmacological treatment appears to be a very successful option.

### • 1416

#### What are we doing in difficult cases (Not closed, Myopic, Large)?

PAPPAS G

*Heraklion*

Macular hole surgery results are generally very good. The closing rate is almost 92%. Patient can gain significant vision. There is though a group of difficult cases. The very large, the myopic macular holes and the redo cases. Traditional and novel techniques will be proposed in order to get the optimal anatomical and functional result.

### • 1417

#### Mechanism of macular hole closure

REPUCCI V

*New York Eye and Ear Infirmary, New York*

Purpose: To outline the mechanism of closure in macular hole surgery; specifically the importance of deforming the fluid-air interface at the edges of the macular hole. This will provide a basis for understanding of how vitrectomy effects macular hole closure. Successful macular hole closure occurs when a fluid interface contacts, and is deformed, by the edges of the hole; generating a "surface of tension" (force), which exceeds the "stiffness" of the retina, allowing for reapproximation of the hole edges along the inner retina. Mechanical barriers, such as posterior hyaloid or vitreous, may reduce the interface deformation and transmittal of these forces to the inner retinal surface; peeling ILM or manipulating the hole edge may reduce the "stiffness" of the retina requiring less force to achieve closure. Larger bubbles increase the macular hole interface contact angle creating interface deformation, and force generation, at the hole edge over a greater range of eye position. Conclusion: Macular holes close by a "bridging" along the inner retinal surface with migration assisted by the capillary forces generated by fluid-air interface deformation at the edges of the macular hole.

**• 1421****Update on ocular surface disorders in aniridia**

BREMOND-GIGNAC D

Amiens

Aniridia is a rare bilateral ocular disorder occurring at a frequency of approximately 1 in 80 000. Aniridia is a panophthalmic disease. About one third of these aniridia are sporadic, with many variable expressivities of the features. PAX6 gene anomalies with 11p13 mutations or deletion are involved in aniridia. PAX6 mutations result in alterations in corneal cytokeratin expression, cell adhesion and glycoconjugate expression. Ocular anomalies in aniridia commonly associates cataract, glaucoma, nystagmus, ptosis and ocular surface. Mechanism of limbal stem cell insufficiency will be exposed and often correlated to reduced corneal sensitivity. Therapeutic options will be discussed.

**• 1422****Update on corneal dystrophies in children**

CHIAMBARETTA F

Clermont Ferrand

**ABSTRACT NOT PROVIDED****• 1423****LSCD - What clues does the infant limbus hold?**

YEUNG A

Division of Ophthalmology and Visual Sciences, Nottingham

Limbal Stem Cell Deficiency (LSCD) is a condition that can cause blindness if left untreated. The current management approach uses a mixture of medical and surgical techniques. We look at the infant limbus for further clues in the management of LSCD.

**• 1424****Update on ocular surface burn in children**

GICQUEL JJ

Poitiers

Children can be the victims of ocular surface burns, due to the exposition to chemical agents, extreme temperatures or ultraviolet, infrared or ionising radiations. Alkali burns differ from acid burns by the fact that they deeply penetrate in the anterior segment and so are particularly severe. Initial clinical examination (with the help of classifications) tells us how to adapt the treatment and gives us the prognosis. The 4 Grades Roppen-Hall's classification has been replaced by the more precise 6 Grades Dua's one. Surgical treatment aiming to restoring a proper corneal epithelium has improved the prognosis of severe cases. It can address the acute (Amniotic Membrane Transplantation, Sequential Sectorial Epitheliectomies) or the late stage of the disease (Limbal Stem Cell (LSC) Transplantation / in or ex vivo expansion). In all cases, an optimal control of the ocular surface inflammation is essential to the LSC survival. In this presentation, we will apprehend the specificities of the management of ocular surface burns in children.

## Course 2: Glaucoma? Yes I can!

### • 1431

#### **My basic slit-lamp? Yes I can!**

*BRON A*

*Dijon*

Before embarking to the prescription and the interpretation imaging techniques it is mandatory to carefully study the personal history of the patient and to record the symptoms and signs during the first steps of the clinical examination. Like the stethoscope for general practitioners and cardiologists, the slit-lamp is a key step of the examination for ocular diseases by ophthalmologists. The details of the medical personal history and the findings collected during the slit-lamp examination will help a lot for the diagnosis and the treatment of glaucoma. In this course, young ophthalmologists will find a check list to help them in retrieving medical history, symptoms and a check list for the slit-lamp examination from the lids to the anterior segment. Nowadays the evaluation of ocular surface for a glaucoma patient will help in choosing the more appropriate topical treatment. This rapid and systematic approach is designed to help young colleagues to improve their daily clinical practice with glaucoma patients.

### • 1432

#### **Gonioscopy? Yes I can!**

*HOMMER A*

*Vienna*

Gonioscopy is an essential examination in Glaucoma care. There is no other technique available that can replace it. Differential diagnosis of the different types of glaucoma is not possible without it. This course will highlight the different lenses, examination technique, findings and classifications. It will be shown with Videos, Computer animation as well as clinical cases.

### • 1433

#### **Clinical examination of the optic disc? Yes I can!**

*JONAS J*

*Mannheim*

The course will present and discuss ophthalmoscopic characteristics of the optic nerve head in normal eyes and in glaucomatous eyes, including gamma zone and delta zone of parapapillary atrophy and histologic findings

### • 1434

#### **Function in glaucoma? Yes I can!**

*ZEYEN T*

*Leuven*

Automated perimetry is the most important functional test in glaucoma patients. This course will teach which programs and strategies to use, how to instruct patients and avoid artifacts, and how to use the available software to interpret worsening of visual field defects and calculate rates of change. Finally, consensus statements will be provided on the usefulness of non-conventional perimetric tests.

**Course 2: Glaucoma? Yes I can!**

• 1435

**Structure in glaucoma? Yes I can!**

*SUNARIC MEGEVAND G  
Memorial de Rothschild, Geneva*

This course is designed for young ophthalmologists and will give the opportunity to discuss aspects of the structure and structural changes of the optic nerve head and the retinal nerve fiber layer related to glaucoma and the alteration of the visual function related to this disease. In the last few years there is increasing evidence of the importance of structural measurements particularly early in the disease, and combining information from structure and function can significantly improve detection and assessment of glaucoma progression. Different tools and new technologies for measuring structural changes will be discussed in details such as stereo- photographs, HRT, GDx and OCT.

**• 1441****Doppler optical coherence tomography***SCHMETTERER L (1, 2)**(1) Clinical Pharmacology, Vienna**(2) Medical Physics and Biomedical Engineering, Vienna*

Doppler optical coherence tomography (OCT) is a functional extension of OCT. In Fourier Domain OCT information on blood flow can be extracted when phase-sensitive measurements are performed. This technique can be used to quantify blood flow but also to visualize the vasculature comparable to angiography. When blood flow in the retina is quantified knowledge on the orientation of the vessel relative to the illuminating laser beam is required. Several attempts have been tried to solve this problem. A promising technique is to illuminate the vessel from two directions with two laser beams. With this approach the extraction of blood velocity becomes independent of incident angle and independent of eye movements. We have shown that this is a valid and reproducible technique to quantify retinal blood flow in humans. When doing angiography systems with high readout rate are required, because of motion artifacts. The technique may have significant potential in retinal disease.

**• 1442****Retinal oximetry methodology***HARDARSON S**University of Iceland / Landspítali, Dept. of Ophthalmology, Reykjavík*

Disturbances in retinal oxygenation are believed to play an important role in several eye diseases, such as retinal vessel occlusions, diabetic retinopathy and retinopathy of prematurity. Non-invasive measurements of retinal oxygenation are important for better understanding of disease mechanisms and the technology will hopefully, in the near future, aid in management of the diseases. The technology for retinal oximetry has been developed in the past years and decades and the current oximeters are sensitive to changes in oxygen saturation and give repeatable results. Studies have confirmed differences in retinal vessel oxygen saturation in various eye diseases. Further studies of retinal oxygenation will benefit from development of the methodology. This includes hardware and software development but also standardisation of measurement techniques with the current instruments and better understanding of confounding factors.

*Commercial interest***• 1443****Determination of macular pigment***BERENDSCHOT T**University Eye Clinic Maastricht, Maastricht*

In 1994 ago Seddon et al. observed an inverse association between a diet with a high content of the carotenoids lutein and zeaxanthin, and the prevalence of age-related macular degeneration. This finding triggered an interest in the macular pigment, since it is solely composed of these two carotenoids. Macular pigment is concentrated in the central area of the retina along the axons of the cone photoreceptors. There are some plausible arguments to assume it exerts a protective effect in the retinal area: It acts as a blue light filter, absorbing between 390 and 540 nm, thereby decreasing chances for photochemical light damage. In addition, macular pigment is capable of scavenging free radicals. In this talk all existing measurement techniques will be discussed to determine the macular pigment optical density and its spatial distribution.

**• 1444****In vivo cell-scale imaging of the human retina by adaptive optics***PAQUES M**Clinical Investigation Center 503, Paris*

Diagnosis and monitoring of retinal diseases, the most important causes of blindness in developed countries, is largely based on accurate imaging of the retina. We have been using a flood imaging, commercially available AO camera (rtx1 from ImagineEye) to examine a variety of retinal diseases. Geographic atrophy can be detected at a very early stage under the form of patches of depigmented RPE filled with numerous clumps of melanin. Time-lapse imaging show the progression of atrophy at a very small spatial and temporal scale, as well as the motion of melanin clumps within and outside atrophic areas. Diabetic microaneurysms can be distinguished from hemorrhages. Epiretinal membranes can be observed under the form of small star-like hyperreflective spots. Finally, parietal remodelling of small arteries following arterial hypertension can be detected, which may provide a novel quantitative biomarker. To sum up, while AO imaging was originally used for photoreceptor imaging, its scope is now extending. AO fundus imaging will probably establish itself as a reference methodology for early detection and monitoring of a variety of retinal diseases.

• 1451

### Pathophysiology of uveitis

DICK A

*School of Cellular and Molecular Medicine, Bristol*

This talk will overview the pathophysiology of non-infectious uveitis in relation to recent SUN (standardised uveitis nomenclature) disease classification. The experimental and translational human evidence of autoimmunity and activation of immunity will be discussed. In addition the talk will highlight the pathways and mechanisms of tissue damage that results in sight-threatening disease. Traditionally, despite active immune regulatory mechanisms operative within the ocular environment, inflammation still occurs. Activated antigen and non-antigen specific T cells are generated in uveitis. The interplay with innate immunity and in particular cells of myeloid lineage both systemically and within the local environment dictate the severity and extent of pathology we observe. The understanding of immune responses during the uveitis open many avenues to potential novel immunotherapies that not only suppress inflammation but attempt to redress immune balance, tolerance and local homeostasis within ocular tissues.

• 1452

### Classification of uveitis

ANDROUDIS

*Thessaloniki*

The use of classification criteria, supported by standardisation guidelines, is very important for disorders that have a multitude of associated aetiologies. The most widely used classification of uveitis is the one devised by the International Uveitis Study Group (IUSG) in 1987, based on the anatomical location of the inflammation. This classification includes anterior uveitis (iritis, iridocyclitis, and anterior cyclitis), intermediate uveitis (pars planitis, posterior cyclitis, and hyalitis), posterior uveitis (focal, multifocal, or diffuse choroiditis, chorioretinitis, retinitis, and neuroretinitis) or panuveitis (anterior chamber, vitreous, retina, and choroid). Today's uveitis nomenclature has been revised regarding the anatomical location and the grade of inflammation, and supplemented by the inclusion of definitions for onset, duration and course. While helpful for clinical practice and clinical trials, further work still needs to be carried out concentrating on validated clinical assessment tools for activity and damage for common, specific uveitic entities.

• 1453

### Signs and symptoms of uveitis

NERI P, CAPUANO V, PIRANI V, ARAPI I, MARIOTTI C, GIOVANNINI A

*The Eye Clinic, Polytechnic University of Marche, Ancona*

Uveitis is a potentially sight-threatening disease and can be categorized into: anterior, intermediate, posterior, and panuveitis. The onset of uveitis can be either acute or insidious, involving one or both eyes. The commonest ocular symptoms are: blurred vision, ocular pain, photophobia and floaters, depending on the type of uveitis. Posterior uveitis is usually associated with vitritis. Anterior chamber cells and flare should be graded according to standardized uveitis nomenclature (SUN) working group. Binocular indirect ophthalmoscopy (BIO) score is used to grade vitreous involvement. Vitreous changes may comprehend: vitreous hemorrhage, vitreous strands, and vitreous traction. A further classification of posterior uveitis depends on the primary site of inflammation, which can identify: retinitis, choroiditis, retinochoroiditis, and chorioretinitis. Posterior pole uveal involvement can be: focal, multifocal, and placoid. Retinal vasculitis can be associated with several sub-types of posterior uveitis. Uveitis can present several complications such as, anterior and posterior synechiae, which can lead to uveitic glaucoma, cystoid macular oedema, retinal and choroidal neovascularizations, and retinal ischemia.

• 1454

### Laboratory work-up and specialized investigations

PLEYER U

*Department of Ophthalmology, Charité, Humboldt University, Berlin*

Based on the anatomical involvement of the eye intraocular inflammation is classified into anterior, intermediate, posterior and panuveitis. All subtypes of uveitis are potentially related to infectious and noninfectious etiologies. This presentation will assist the participants in accurately diagnosing uveitis in a step latter approach including physical and laboratory investigations. In addition, a tailored approach based on confounding clinical observations with specialized investigations will help to further differentiate clinical entities. In cases of suspected intraocular infections the option of intraocular fluid evaluation for antibody testing and polymerase chain testing against the causative agent will be presented. Taken together, this part of the course will provide a rational decision-making strategy for diagnosis of patients with uveitis.

### Course 3: EBO review course: Intraocular Inflammation and Infection - Part I

#### • 1455

#### **Imaging in uveitis: techniques and indications**

HERBORT C (1, 2)

(1) Centre for Ophthalmic Specialized Care, Lausanne

(2) University of Lausanne, Lausanne

This tutorial will address the main complementary imaging techniques used in the field of (posterior) uveitis. In case imaging work-up is decided, fluorescein angiography (FA) is performed routinely since a few decades. FA gives information on the superficial structures and lesions of the fundus including pathology of the retina, retinal vessels, optic disc, and subretinal fluid collection, as well as the RPE for which it is the examination method of choice, and the choriocapillaris in the first seconds of angiography. Most of the time it only confirms and gives the precise extension of lesions already identified by the clinical examination. The choroid is however involved at least as often as the retina and often all or part of choroidal lesions are occult and not detected by the clinical exam or FA. Only indocyanine green angiography (ICGA) gives visual access to choroidal inflammatory pathology where it can distinguish stromal choroiditis (birdshot, VKH) from choriocapillitis (MEWDS, APMPPF, etc). So if angiography is deemed necessary during initial appraisal of a case dual FA/ICGA should be performed as choroiditis can not be excluded "à priori". Other methods addressed will be OCT, UBM, FAF as well as anterior segment OCT.

#### • 1456

#### **Therapeutic management of uveitis**

DICK A

School of Cellular and Molecular Medicine, Bristol

This talk will overview the contemporary therapeutic approaches to treatment of non-infectious non-infective ocular inflammatory disease. Treatment of non-infectious uveitis has over past 15 years expanded from the use of traditional therapies including corticosteroids and immunosuppressants to the deployment of targeting the immune response with biologic therapies with monoclonal antibodies and immunoadhesins. Such use will be exemplified with case reports during the talk. Evidence of efficacy of immunosuppressants in the treatment of uveitis, the role of predicting steroid responsiveness, the use of monotherapy with immunosuppression and finally the pathways and evidence of success of biologic therapy will be provided.

• 1461

**The initial consult**

SPILEERS W

*Dept. of Ophthalmology, University Hospitals, Leuven*

Patients with involuntary eye movements frequently consult an ophthalmologist. A correct diagnosis of the type and etiology is important. A clinical approach with selected technical examinations is mandatory for a correct diagnostic approach. Isolated ophthalmological and combined neurological elements can be identified to guide the further approach. Conclusions A practical work-up of a patient presenting with nystagmus will be presented. Frequently the collaboration with the neurologist is important.

• 1462

**Genetically determined causes of eye movement disorders**

LEROY B

*Ghent*

To describe the phenotypes and genotypes of genetically determined disease leading to nystagmus. A case presentation format will be used to illustrate different genetically determined conditions leading to nystagmus. Both clinical and electrophysiological phenotypes as well as genotypes will be discussed. Phenotypes and genotypes of genetically determined nystagmus are very different. An important distinction to be made is the one between stationary and progressive diseases. Indeed, such distinction is important as the visual outcome varies considerably between those different conditions. Very diverse conditions may give rise to genetically determined nystagmus. Genetics and visual electrophysiology allows an important distinction between progressive and stationary conditions

• 1463

**The neurological approach**

KAWASAKIA

*Lausanne*

Abnormal eye movements with or without subjective oscillopsia may be a the first manifestation of serious ocular or intracranial pathology or toxicity. This talk will focus on identifying those in which early diagnosis and/or treatment is important in the overall prognosis of the patient.

• 1464

**The role of electrophysiology**

HOLDER G

*Moorfields Eye Hospital, London*

The presentation will utilise a case-based approach to address management issues in the patient with involuntary eye movements. Accurate prognostic information can only be given when an accurate diagnosis has been established. Electrophysiological examination facilitates diagnosis, and helps distinguish between stationary and progressive disorders, and those disorders which are relatively benign from progressive blinding disorders. Characteristic ERG changes can also enable appropriate genetic screening even in patients with atypical clinical features.

**Joint Meeting: FRO - Belgian Fund for Research in Ophthalmology - part 1**

• 1471

**The effect of local rock-inhibition on uveitis**

*HOLLANDERS K  
Leuven*

**ABSTRACT NOT PROVIDED**

• 1472

**Identification of the gene signature of retinal endothelial cells during classical experimental autoimmune uveitis, Th1- and Th17-dependent uveitis**

*LIPSKID*

**ABSTRACT NOT PROVIDED**

• 1473

**Evaluating hyaluronic acid as a coating strategy for intravitreally injected nanomedicines for retinal gene therapy**

*MARTENS T  
Ghent*

**ABSTRACT NOT PROVIDED**

• 1474

**Examining the optical qualities of explanted IOLs**

*NIDHUBHGHAILLS*

**ABSTRACT NOT PROVIDED**

**• 1475**

**Development of a classification system for normal and keratoconic human corneas to assist in the detection of early keratoconus through a machine learning algorithm**

RUIZ HIDALGO I

**ABSTRACT NOT PROVIDED**

**• 1476**

**Potential implications of epigenetic changes in human retinal pigmented epithelium during diabetic retinopathy**

SALIK D  
Bruxelles

**ABSTRACT NOT PROVIDED**

**• 1477**

**Complementary effects of mitomycin-c and anti-fibrotic agents on surgical outcome after glaucoma filtration surgery**

VAN BERGEN T  
Leuven

**ABSTRACT NOT PROVIDED**

**• 1478**

**Endothelial recovery by novel rock-inhibitors in the cornea**

SIJNAVE D  
Leuven

**ABSTRACT NOT PROVIDED**

**Industry-sponsored symposium 1: Glaucoma and the Ocular Surface****• 1541****Glaucoma and the ocular surface***BRONA**Dijon***ABSTRACT NOT PROVIDED****• 1542****How to recognize and decide if a patient suitable for PF therapy***GONIF**Barcelona***ABSTRACT NOT PROVIDED****• 1543****PF Monotherapy***ROSSETTI L**Milano***ABSTRACT NOT PROVIDED****• 1544****PF Combo therapy***SUNARIC MEGEVAND G**Geneva***ABSTRACT NOT PROVIDED**

**• 1611****Dietary fat consumption and AMD: Epidemiological facts**

DELCOURT C (1, 2)

(1) Univ. Bordeaux, Isped, Bordeaux

(2) Inserm, Centre Inserm U897 Epidemiologie-Biostatistique, Bordeaux

In 2000, a 50 % lower risk for AMD in regular fish consumers was reported for the first time, in a cross-sectional analysis of the Blue Mountains Eye Study. Since then, 8 cross-sectional and case-control studies have produced similar results, although not always reaching statistical significance. However, cross-sectional and case-control studies are subject to reverse causality: subjects with AMD may have decreased their fish consumption because of the disease itself, for instance because of difficulties in shopping and cooking. Six prospective studies, in which dietary habits are assessed before the onset of AMD, have also all shown a reduced risk for AMD in high consumers of fish or omega 3 LC-PUFA, although not always reaching statistical significance. Finally, use of biomarkers may help overcome many methodological difficulties of dietary assessment (recall bias, imprecisions in assessment of food intake and nutritional content of foods...). In the French Alienor Study, we have recently shown that subjects with high plasma omega3 LC-PUFA had a lower risk of AMD within the next 10 years. Overall, epidemiological data are strikingly consistent, showing a major reduction of risk for AMD in subjects with high omega3 LC-PUFA status.

**Commercial interest****• 1612****Omega-3 fatty acids and prevention of cardiovascular events**

DALLONGEVILLE J

Lille

Linoleic acid (LA) and alpha-linolenic acid (ALA) are precursors of omega-6 and omega-3 fatty acids. The effects of omega-3 on cardiovascular risk factors – such as lipoprotein levels, blood pressure and glycemia – are very modest at standard low doses, significant only for large intakes. The observation of food habits in population cohorts allowed to define the effects of rich in ALA, EPA, DHA on the risk of myocardial infarction, coronary heart disease and sudden death. For ALA the results are inconsistent. Randomised trials have shown that oils enriched in EPA and DHA have an undeniable impact on heart rhythm. Consumers of long-chain fatty acid have lower heart rates than controls. The meta-analysis of cohort studies shows that consumption of omega-3 long-chain (EPA-DHA) reduces total mortality, death from cardiac causes, sudden death and possibly stroke. The data from prevention trials with omega-3 long-chain showed a decrease in coronary events, including fatal, in secondary prevention. These results are relatively consistent to believe that EPA and DHA intake reduces the fatal complications of myocardial infarction. In the present talk the most recent results of clinical trials on the prevention of CVD events by omega-3 fatty acids will be presented.

**• 1613****The mechanisms of the action of omega-3s in the retina**

ACAR N

Eye and Nutrition Research Group, INRA, Dijon

Age-related Macular Degeneration (AMD) is the leading cause of visual loss in Western countries after the age of 50y. Based on large-scale epidemiologic studies, it appears now as evident that omega-3 polyunsaturated fatty acids (PUFAs) provide benefits in preventing both, early and late stages of AMD. The aim of this paper is to summarize the knowledge about the biological mechanisms by which omega-3 PUFAs may be protective for the retina. The content of this presentation will range from biochemical data about the occurrence of omega-3 PUFAs in retinal cell membranes to results from cellular and animal studies showing that omega-3 PUFAs can influence processes involved in signal transduction, oxidative stress, apoptosis or inflammation.

**• 1614****Prospects: Dietary  $\omega$ -3 polyunsaturated fatty acids, CCR2+ monocyte recruitment, and AMD?**

SENNLAUB F

Institut de la Vision, Paris

Oxidative stress is thought to be an important player in the development of atrophic AMD. Chronic photo-oxidative stress induced phospholipid oxidation increases the expression of C-C motif chemokine 2 (CCL2), which is known to recruit CCR2+ monocyte in inflammatory process. Their possible role in promoting or inhibiting retinal degeneration is unknown. We show that atrophic AMD is associated with increased intraocular CCL2 levels and subretinal CCR2+ inflammatory monocyte infiltration in patients. Using age- and light-induced subretinal inflammation and photoreceptor degeneration in Cx3cr1 deficient mice, we show that genetic deletion of CCL2 or CCR2 and pharmacological inhibition of CCR2 prevents inflammatory monocyte recruitment, mononuclear phagocyte accumulation, and photoreceptor degeneration in vivo. Dietary  $\omega$ -3 polyunsaturated fatty acids (PUFA) have been shown to inhibit CCL2 induction and inflammation in endotoxin-induced uveitis. They might represent a powerful tool for controlling inflammation and neurodegeneration in AMD.

SIS: Dietary fatty acids: from mechanisms to epidemiology and clinical application in the prevention of Age-related Macular Degeneration (AMD)

• 1615

How to handle the dietary requirements in clinical practice in ophthalmology

*CREUZOT C  
Dijon*

**ABSTRACT NOT PROVIDED**

**Course 4: The amniotic membrane: new regenerative therapies and their potential impact on the clinical management of ocular surface diseases****• 1621****Amniotic membrane transplantation: Implications for corneal wound healing***GICQUEL JJ (1), DUA HS (2)**(1) Poitiers**(2) Nottingham***ABSTRACT NOT PROVIDED****• 1622****Intra and inter donor variations of amniotic membrane***GICQUEL JJ**Poitiers*

There is a considerable variation in growth factors content of the Amniotic Membrane, between and within donors. This is further affected by handling of the AM. Such variations could affect the clinical efficacy of tissue constructs. Current use of AM for ex vivo expansion or surgery is not standardized and remains an area of concern. In this course we will apprehend the various aspects influencing these intra and inter-donor variations.

**• 1623****The new clinical applications of dried amniotic membrane***HOPKINSON A, ALLEN C, DUA HS**Division of Ophthalmology & Visual Sciences, Nottingham*

Dried amniotic membrane (AM) can be a useful therapeutic adjunct in ophthalmic surgery and possesses logistical advantages over standard cryopreserved AM. Differences in preservation techniques can significantly improve biochemical composition and physical properties of AM, potentially affecting clinical efficacy. Nottingham have developed a novel dry AM preparation comparable in biochemistry to fresh membrane and with superior in vitro wound healing capability than conventional cryopreserved and freeze dried AM. This product is stable and easily transportable allowing it to be globally wide reaching for use in clinical and military sectors, and can be stored as a stock item. Allowing such an 'off the shelf' application, creates a novel standardised biological dressing in emergency situations for enhanced wound healing and improved clinical prognosis. Their pre-clinical data generated from treatment of ocular burns demonstrates the benefit of acute phase innervation with an effective treatment such as dry AM.

**• 1624****Challenges in manufacturing an amniotic membrane alternative for corneal regeneration***WILSON S, HOPKINSON A**Division of Ophthalmology in Visual Sciences, University of Nottingham, Queen's Medical Centre, Nottingham*

The current and projected worldwide cornea shortages have acted as a driver for the development of feasible, long-term alternatives to cadaveric donor tissue. Alternatives in current clinical or pre-clinical development can be loosely categorised into the following broad areas: keratoprostheses, tissue engineered constructs, xenografts and the use of decellularized/acellular matrices. With respect to corneal regeneration, there are many challenges to address, not least that the corneal structure is unique and difficult to replicate. When manufacturing corneal tissues, the choice of material is a vital consideration as the list of requirements is extensive. They must be biocompatible, (preferably) optically transparent, flexible, and strong, as to withstand manipulation in culture, potential suturing, irrigation and handling during surgery. Furthermore, the manufacturing process needs to be simple with consistent quality, preferably at high speed and low cost. The biodegradability or bioreabsorbility of the construct needs consideration to ensure cellular integration, regeneration and reconstruction. Two fundamental objectives concerning corneal regeneration are the maintenance of healthy cell phenotypes either *in vitro* or *in vivo* following implantation; and the replication of the native tissue architecture. If both factors are not satisfied, the result is often regenerated tissue mimicking that of scarred native tissue.

**Course 4: The amniotic membrane: new regenerative therapies and their potential impact on the clinical management of ocular surface diseases****• 1625****Amniotic membrane as a tissue engineering substrate***BRANCH M**Nottingham*

Amniotic membrane (AM) is commonly used for cell culture and transplantation onto the ocular surface. AM serves the dual purpose of culture substrate and scaffold thus reducing cell manipulation. Crucially AM is believed to promote the retention of stem cell phenotypes essential for the long term restoration of functional tissue. As potential cellular therapies for corneal regeneration are being developed many of them continue to use AM. Its widespread usage in ophthalmology and extensive list of wound healing attributes has made AM a popular choice. Amniotic membrane is being used as a carrier for innovative research into the transplantation of non-corneal cell types onto ocular surface burn models including mesenchymal stem cells and oral mucosal epithelium. A lack of standardisation in the preparation of amniotic membrane can cause considerable variation and some methods of preparation may reduce or enhance certain attributes. Various methods of processing including cross-linking, denuding and freeze drying have sought to enhance amniotic membrane for tissue engineering purposes with varied levels of success.

• 1631

**Healing of a resistant corneal neurotrophic ulcer using a new matrix therapy agent (RGTA)**

PISON A, HAYA DETHOREY G, BREZINA, BOURGES JL  
Hotel-Dieu-Cochin Hospital, Paris

**Purpose** To report the case of a patient with a corneal neurotrophic ulcer, resistant to the usual therapies, who has been treated with a new matrix therapy agent (RGTA). CACICOL 20° is a new ophthalmic device, derived from RGTA based matrix therapy (large biopolymers engineered to replace heparan sulfates).

**Methods** We report the case of a monophtalm and deaf-mute 48 years old woman, who presented with a central neurotrophic corneal ulcer, not responding to the first line therapies, including high dose lubricant eye drops and amniotic membrane graft. To avoid the profound confinement induced by tarsoraphy, a compassionate treatment with RGTA CACICOL 20° has been proposed. CACICOL 20° was instilled once every other day for 2 weeks. Biomicroscopic pictures were recorded to follow the cicatrization time course.

**Results** Epithelialization was obtained within 2 weeks with a perfect tolerance. The regimen of instillation was incidently doubled, causing a recurrence, which healed within 2 weeks after the correct regimen was reintroduced.

**Conclusion** This case report is consistent with a strong effect of CACICOL 20° in the treatment of resistant neurotrophic corneal ulcers. The optimal posology and the indications needs to be refined.

• 1632

**New matrix therapy in chronic corneal ulcers resistant to conventional therapies**

HUGNY LARROQUE C, DERRIEN S, COCHENER B  
Ophthalmologie, Brest

**Purpose** To determine the efficacy of a new ophthalmologic solution based on ReGeneraTing Agent technology (RGTA, Cacicol°) in the therapy of chronic corneal ulcers resistant to conventional therapies.

**Methods** This study included 20 patients with chronic corneal ulcers. There were various etiologies: two due to basic chemical burns, two neurotrophic keratitis, one Moreen ulcer, one pseudo-Mooren, three on IntraCorneal Ring, nine on 9 grafts (one endothelial and 8 penetrating keratoplasties, one post surgery). All patients were previously treated with classical lacrimal substitutes, one with topical ciclosporine, corticoids, and/or A vitamin ocular ointment, and/or one/several amniotic membrane graft. Patients were treated with Cacicol° at a dose regimen of one drop daily every 2 or 3 days for one to three months. The primary outcome measure was healing. The ulcer's diameter was measured at day 8, then once a month for 3 months.

**Results** Complete healing was observed for 13 patients, i.e. a cure rate of 65 %. For other patients, 6 failures (30%) and one improvement without complete healing (5%) were reported. In the healed group, time of reparation was : 4 in day 8, 4 in one month and 5 in 3 months.

**Conclusion** Cicicol was effective in the treatment of chronic corneal ulcers resistant to conventional therapies. Its efficacy remains to be proven in randomized double-blind studies.

• 1633

**A RHO kinase inhibitor, AMA0526 improves corneal wound healing after alkali burn injury**

SIJNAVE D (1), HOLLANDERS K (1), VAN BERGEN T (1), VAN DE VELDE S (1),  
VANDEWALLE E (2), MOONS L (3), STALMANS I (1, 2)

(1) KU Leuven, Ophthalmology, Leuven

(2) UZ Leuven, Ophthalmology, Leuven

(3) Department of Biology, KU Leuven, Leuven

**Purpose** The aim of this study was to investigate the efficacy of a locally active ROCK-inhibitor AMA0526 (Amakem NV) on corneal wound healing induced by alkali burn in an *in vivo* mouse model.

**Methods** Swiss mice were divided randomly into 3 groups after chemical cauterization of the cornea by alkali. Topical treatment was initiated after the injury and given once daily. All groups received 0.1% of the active ROCK-inhibitor AMA0526 topical in one eye and vehicle, bevacizumab (2.5%) or dexamethasone (0.1%) in the contralateral eye. A forth group received no treatment and was used as control. Corneal opacity and corneal neovascularization were graded every other day according to a 0-4 scoring. Blood vessel formation and corneal leukocytes infiltration after injury were assessed by immunohistochemistry.

**Results** Both corneal opacity and neovascularization were reduced after AMA0526 treatment compared to the vehicle treated eye. Infiltration of inflammatory cells and blood vessel formation were significantly inhibited at day 7. AMA0526 treated mice showed significant reduced corneal opacity and inflammation compared to mice treated with bevacizumab. AMA0526 proved to be as efficient as dexamethasone in reducing excessive corneal wound healing.

**Conclusion** Targeting ROCK with a local ROCK inhibitor, AMA0526 is efficacious in improving and preventing corneal opacity and neovascularization after alkali burn. The results presented indicate that ROCK is an appealing target to treat and prevent corneal scarring and neovascularization and illustrate the potential therapeutic benefits of the local ROCK inhibitor, AMA0526.

• 1634

**Diagnostic traps based on nomenclature of inflammatory changes in corneal confocal microscopy *in vivo***

SMEDOWSKI A, WYLEGALA E, WOJCIK L

Ophthalmology Clinic, District Railway Hospital, Katowice

**Purpose** To present possible misdiagnosis of infectious keratitis in corneal confocal microscopy *in vivo* images based on typically used nomenclature.

**Methods** Retrospective analysis of *in vivo* confocal microscopy images from 155 patients with different diagnosis. 63 cases were diagnosed in confocal microscopy as various infectious inflammations and confirmed microbiologically or based on treatment response. 25 of them were recognized as bacterial, 25 as viral, 11 as fungal and 2 as Acanthoamebal keratitis. For each type of inflammation we selected "key words" typically used for describing characteristic corneal changes. In next step we analyzed other, non-inflammatory cases and selected images which could be described with nomenclature using typically for inflammatory changes describing (for example "cyst," "fibers," "round cells infiltration," "dendritic/Langerhans cells presence," etc.).

**Results** From 92 non-inflammatory cases, 35 could be described with inflammatory "key words." These cases represented 14 different pathologies: Cogan and Messmann dystrophy, stroma fibrosis, corneal transplant rejection, in-growth epithelial syndrome, crystal and vortex kerathopathy, after LASIK and after PRK changes, neurotrophic ulcer, superficial nerves outgrowth, diffuse anterior lamellar inflammation after LASEK, ichthyosis and Thygeson inflammation.

**Conclusion** Diagnostic process using confocal microscopy examination cannot be based just on algorithmic analysis of corneal images. For reliable diagnosis microscopy images should be compared with clinical state and anamnesis.

## Free papers COS 1/5: Corneal inflammation and wound healing

### • 1635

#### Short-time reproducibility of tearfilm osmolarity measurement assessed with electrical impedance

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(1) Department of Clinical Pharmacology, Vienna

(2) Center for Medical Physics and Biomedical Engineering, Vienna

(3) Department of Ophthalmology and Optometry, Vienna

**Purpose** There is compelling evidence that increased tearfilm osmolarity is a key pathogenetic mechanism for the development of Dry Eye Syndrom (DES) and its related complications. The current study was performed to assess the short-time and day-to-day reproducibility of an electrical impedance based system for the measurement of tear film osmolarity.

**Methods** 20 patients with moderate DES and a control group of 20 age and sex matched healthy subjects were included in the study. Tear film osmolarity was measured using a commercially available electrical impedance based "lab on a chip" system (TearLab OcuSens). To measure short-time reproducibility 3 consecutive measurements were performed. To assess day-to-day reproducibility this procedure was repeated on three consecutive study days. Schirmer I test and break up time was performed on each study day. Coefficient of variation (CV) was calculated as a measure of reproducibility.

**Results** Tear film osmolarity was higher in patients with DES compared to healthy control subjects on all three study days ( $p<0.05$ ). Short time CV of tearfilm osmolarity on the three study days was  $3.18\% \pm 2.8\%$ ,  $2.81\% \pm 2.08\%$  and  $2.23\% \pm 1.85\%$ , respectively. Day-to-day variability based on the CV of the three study days was  $3.47\% \pm 1.69\%$ . As expected, Schirmer I test and BUT was lower in patients with DES compared to healthy subjects on all three study days ( $p<0.05$ ).

**Conclusion** Electrical impedance based measurements of tear film osmolarity show good short time and day-to-day reproducibility. Whether tear film osmolarity is a suitable clinical parameter to assess severity and to monitor treatment success in patients with DES has yet to be investigated.

### • 1636

#### Corneal Langerhans cell and dry eye examinations in ankylosing spondylitis

MARSOV/SZKY L (1), NEMETH J (1), RESCH MD (1), TOLDI G (2), LEGANY N (3), KOVACS L (3), BALOG A (3)

(1) Semmelweis University, Department of Ophthalmology, Budapest

(2) Semmelweis University, First Department of Pediatrics, Budapest

(3) University of Szeged, Department of Rheumatology, Szeged

**Purpose** To examine the density and the distribution of corneal Langerhans cells (LCs) and to compare the results with dry-eye related parameters and disease activity in ankylosing spondylitis(AS).

**Methods** Twenty four AS patients (mean age:  $41.8 \pm 9.8$  years) with various degree of disease activity and twenty four healthy subjects (mean age:  $47.9 \pm 16.4$  years) were enrolled. Ocular surface disease index (OSDI), lid parallel conjunctival folds (LIPCOF), tear break up time (TBUT), and Schirmer test (ST) were evaluated. In addition, central and peripheral LCs numbers and Langerhans cell morphology (LCM) were assessed with in vivo laser confocal microscopy.

**Results** Tear production was greatly suppressed in patients with more severe systemic inflammation according to the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and C-reactive protein (CRP) (BASDAI $\leq 4.0$  vs BASDAI $> 4.0$   $10.2 \pm 8.5$  vs  $4.0 \pm 5.3$ ; CRP $\leq 5.0$  vs CRP $> 5.0$   $17.2 \pm 4.2$  vs  $3.9 \pm 5.5$   $p < 0.05$  for all). LCs densities and central LCM were greater in AS patients than in the controls (LC density:  $77.5 \pm 44.9$  vs  $23.8 \pm 33.8$  and central LCM:  $1.7 \pm 0.7$  vs  $0.95 \pm 0.75$   $p < 0.05$  for all).

**Conclusion** Greater corneal LC density and LCM may reflect an increased activation of the corneal innate immune system, which correlates with the systemic activity of AS even without ocular symptoms. Higher systemic inflammation might impair tear production, and it might partly explain the dry eye mechanism.

### • 1637

#### Experimental evidence of fluid secretion of rabbit lacrimal gland ductal epithelia

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(1) Dept. of Ophthalmology, Szeged

(2) 1st Dept. of Internal Med., Szeged

(3) Dept. of Pharmacology, Szeged

(4) Dept. of Pharmaceutical Chemistry, Szeged

**Purpose** Authors developed a novel lacrimal gland (LG) duct isolation technique earlier (IOVS, 2007; 48:3746-3755) which makes possible to obtain more information on the function and regulation of LG ductal cells. The role of LG ductal epithelium on fluid secretion is unknown. The aim of the present study was to investigate LG ductal fluid secretion by means of measurement of fluid secretion evoked by different agonists.

**Methods** Experiments were performed on isolated rabbit LG interlobular duct segments maintained in short term culture. The ends of the ducts sealed after overnight incubation forming a closed luminal space. Fluid secretion into closed intraluminal space as swelling response was analyzed using bright field videomicroscopy technique.

**Results** The secretory response to carbachol or to forskolin stimulation was measured in HEPES buffered and in HCO<sub>3</sub><sup>-</sup>/CO<sub>2</sub><sup>-</sup> buffered solutions. Carbachol initiated ductal swelling. This result proves the cholinergic effect-evoked ductal fluid secretion. Fluid secretion was biphasic consisting of a continuous swelling in the first 5 minutes followed by a plateau phase. Secretory rates did not show a substantial difference measured in HEPES and HCO<sub>3</sub><sup>-</sup>/CO<sub>2</sub><sup>-</sup> buffered solutions. In contrast to the biphasic effect of carbachol, forskolin stimulation resulted in a rapid, continuous swelling response. The secretory effect of forskolin did not differ in HEPES and HCO<sub>3</sub><sup>-</sup>/CO<sub>2</sub><sup>-</sup> buffered solutions.

**Conclusion** Fluid secretion of LG ductal cells induced by carbachol and by forskolin stimulations strongly supports the hypothesis, that LG ductal system is actively involved in lacrimal fluid secretion. Our results provide the first experimental evidence of secretory function of this epithelium.

**• 1641****Pharmacological strategies related to ocular neuroprotection***GARHOFER G**Vienna*

Ocular neuroprotection is a novel approach in the treatment of eye diseases, aiming to prevent or at least slow down loss of retinal neurons to avoid disease progression. Although ocular neurodegenerative diseases differ in their pathogenetic properties and risk factors from cerebral diseases, mechanisms behind neurodegeneration and cell death show similarities. As such, several new anti-apoptotic agents, targeting the different mechanisms to induce neuronal cell death have been introduced in the last years. These mechanisms include but are not limited to excitotoxicity, oxidative stress and subclinical inflammation. From a clinical side of view the most commonly used target for neuroprotective therapy in the past was glutamate excitotoxicity. As glutamate excitotoxicity is among the most important mechanisms to trigger cell death, much emphasis has been put into the development of glutamate antagonists. However, the clinical results of interventions studies fell short of expectations and other strategies came into focus of research. The talk aims to summarize the most promising pharmacological neuroprotective strategies.

**• 1642****Importance of taurine in maintaining retinal function***PICALID S (1, 2, 3), FROGER N (4)**(1) INSERM, Institut de la Vision, Paris**(2) UPMC Univ Paris 06, Paris**(3) CNRS, UMR\_7210., Paris**(4) Institut de la Vision, Paris*

Taurine depletion has been known to induce photoreceptor degeneration since the 70s. Recently, investigating the retinal toxicity of the antiepileptic drug, vigabatrin, we showed that taurine depletion causes retinal ganglion cell loss. Indeed, the primary site of vigabatrin-induced injury appears to be located in retinal ganglion cells in patients. The taurine depletion was demonstrated in both vigabatrin-treated animals and patients. In fact, an increase in intraocular pressure as in glaucoma could also induce a local retinal taurine depletion. Taurine supplementation was neuroprotective in 4 animal models with retinal ganglion cell loss. Taurine acts directly on retinal ganglion increasing their survival in pure cultures. Taurine also reduces their glutamate excitotoxicity in retinal explants. Therefore, taurine is required for the normal maintenance of photoreceptors but also for the maintenance of retinal ganglion cells. Taurine supplementation may therefore provide a novel neuroprotective strategy for retinal diseases like glaucoma.

**• 1643****Clinical neuroprotection in relation to ophthalmology***ROSSETTI L**University Eye Clinic, San Paolo Hospital, Milano*

Neuroprotection has been proposed as a possible therapeutic paradigm for the treatment of glaucoma. Unfortunately, despite the bulk of evidence coming from experimental animal research, very few data on effective neuroprotection in human glaucoma are available. A first problem is related to clinical trials on neuroprotection: the choice of study design, types of patients and study outcomes has often been debated. Then the question of the right compound is also a fundamental issue. As of today, to carry out a clinical trial potentially providing convincing evidence of neuroprotection in glaucoma is considered so expensive that no company would think to put money in it. On the other hand, there are some small studies showing surprisingly large effects on various outcomes relevant for glaucoma. Looking at the existing examples of clinical trial on neuroprotection in glaucoma (e.g. the memantine trial, the LoGTs, etc.), better designed studies should be proposed and research to validate new outcomes highly encouraged.

**• 1644****Possible reasons for a miss match between animal neuroprotection studies and clinical findings***OSBORNE N (1, 2)**(1) Fundación de Investigación Oftalmológica, Oviedo**(2) Nuffield Dept.Clinical Neurosciences, Oxford*

A variety of reasons exist to possibly explain why excellent experimental neuroprotection studies have not been translated to a functional attenuation of a defined progressive retinal disease like glaucoma. A major problem exists in judging the validity of published experimental studies. For example, it is questionable how the various animal models used really relate to any specific ocular disease. There is also a tendency to accept studies as being correct when reported in high profile journals and/or from leading groups. Another issue is that methods used for the administration of neuroprotectants that relate to acceptable side effects, penetration, pharmacokinetics and therapeutic efficacy in successful animal studies might not translate in the same way to the human situation. Also, most animal studies do not demonstrate functional neuroprotection but rather the preservation of biochemical, physiological or morphological alterations caused by a defined insult. It should however be pointed out that clinical proof to reproduce experimental studies in relation to ocular neuroprotection remains largely unexplored because of costs and where investigated may have been faulty.

## Course 5: EBO review course: Intraocular Inflammation and Infection - Part II

### • 1651

#### B27-associated uveitis, Fuchs uveitis

WILLERMAIN F

Bruxelles

B27-associated uveitis is a very frequent form of non infectious intraocular inflammation which account for approximately 50 % of acute anterior uveitis. Its main clinical features, natural history and association with seronegative arthritis are well known. Fuchs uveitis is another frequent cause of anterior and intermediate uveitis. Its natural history is well characterised as well as its association with intraocular production of anti-rubella antibodies. Both diseases are thus often considered as easy diagnosis. However, several aspects of those diseases remain challenging and debated. In this interactive course, based on clinical cases, we will insist on those difficult aspects as well as on the more recent issues discussed in the literature.

### • 1652

#### Infectious uveitis

PLEYER U

*Department of Ophthalmology, Charité – University Medicine Berlin, Berlin*

The differential diagnosis of infectious uveitis is broad and an essential step in any initial work-up. Underlying organisms include all types of infectious agents. The more common infectious causes of uveitis include viral causes, toxoplasmosis, syphilis, tuberculosis and endogenous endophthalmitis that will be covered in this course. Based on clinical features further diagnostic tools will be discussed and critically reviewed. In particular newer evolving techniques in the investigations will be included, e.g. intraocular fluid evaluation for polymerase chain testing for the genome and antibody testing against the causative organisms.

### • 1653

#### Behçet disease, VKH, sarcoidosis

KHAIRALLAH M (1), KAHLOUN R (2)

(1) Monastir

(2) Ophthalmology, Fattouma Bourguiba University Hospital, Monastir

Ocular involvement associated with Behçet disease is characterized by a relapsing remitting panuveitis with diffuse vitritis, retinal infiltrates, and occlusive vasculitis. Proper management relies on the early use of immunosuppressive drugs in combination with corticosteroids and administration of biologic agent in resistant and severe posterior segment involvement. VKH disease is a bilateral panuveitis that may be associated with extraocular manifestations. Exudative retinal detachment, associated with typical imaging findings, is the most specific feature to acute VKH disease. Sunset glow fundus is typical to chronic VKH disease. Complications are more likely to occur in the chronic recurrent phase. The mainstay of treatment for acute VKH disease relies on systemic corticosteroid therapy for at least 6 months. Immunosuppressive therapy is mainly used in chronic recurrent disease. Main ocular features of sarcoidosis include bilateral granulomatous anterior uveitis, vitritis with snowballs, multifocal chorioretinitis, and segmental periphlebitis. Diagnosis may be challenging in the absence of apparent systemic involvement. Treatment of sarcoidosis is based on corticosteroids and immunosuppressive agents, in severe cases.

### • 1654

#### White dot syndromes

HERBORT C (1, 2)

(1) Centre for Ophthalmic Specialized Care, Lausanne

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"White dot syndromes" (WDS) is a term introduced around 1995 to describe posterior uveitis syndromes that were poorly understood such as MEWDS, APMPE, multifocal choroiditis (MFC), serpiginous choroiditis (SC), birdshot retinochoroiditis (BRC) and many others depending on the extension with which the term is used. Unfortunately the term is of no utility as it is purely based on the white dots most posterior uveitis exhibit and as it encompasses entities that look alike but have nothing in common as far as mechanism is concerned. Thanks to indocyanine green angiography (ICGA) it became possible to get away from this pot-pourri terminology and allowed to sort out choroiditis entities according to the pathophysiological mechanism subdividing choroiditis into diseases of the choriocapillaris (primary choriocapillitis) including MEWDS, APMPE, MFC, SC and atypical and overlapping entities on one side and stromal choroiditis on the other side including Vogt-Koxanangi-Harada disease (VKH), BRC, sarcoid and tubercular choroiditis. The appraisal of these diseases and the rationale of their new classification will be explained and examples will be given to illustrate this new comprehensive approach that should make WDS obsolete.

**• 1655****Pediatric uveitis***BODAGHIB**Paris*

The etiology and treatment of uveitis in children remains different from adults. Infectious and auto-immune conditions must be identified. Juvenile idiopathic arthritis-associated uveitis is the main etiology of chronic anterior uveitis. Pars planitis is another frequent etiology of bilateral auto-immune uveitis. On the other hand, toxoplasmic retinochoroiditis, ocular toxocariasis and cat scratch disease should be excluded in children with unilateral posterior uveitis. Case reports will be presented in order to illustrate the management of different pediatric uveitis entities.

## Free papers EOVS 1/1: Visual function and neurophysiology

### • 1661

#### Motion-onset visual evoked potentials - Important tool in vision and eye research

KUBOVA Z, KUBA M, KREMLACEK J, LANGROVA J, SZANYI J, VIT F, CHUTNA M  
Charles University - Faculty of Medicine, Hradec Kralove

**Purpose** Despite quite a long history of visual evoked potentials (VEPs), their use has been limited so far almost exclusively to flash or pattern related responses of the primary visual cortex (mediated mainly via activation of the parvocellular system of the visual pathway. It is surprising that efforts to extend it to more complex testing including an examination of the motion processing system (magnocellular system and dorsal stream) are quite rare (e.g. Kuba and Kubová, Doc Ophthalmol, 1992, 80, 83-89; Kubová et al., 1995, Vision Res, 35, 197-205).

**Methods** Early or selective involvement of the motion processing system is suspected in many CNS disorders and we suggest that motion-onset VEPs may recognize functional problems of this kind better than imagine techniques (Kuba et al., Vision Res, 2007, 47, 189-202).

**Results** Motion-onset VEPs display latency shortening up to the age of about 18 years representing very slow individually different maturation of the motion processing system (Langrova et al., Vision Res., 2006, 46, 536-544. In early adulthood they start accelerated latency prolongation when compared to pattern-reversal, which might be a good indicator of individual biological ageing (Kuba et al., Vision Res, 2012, 62, 9-16.

**Conclusion** Since about 30% of pathological findings in our parallel pattern-reversal and motion-onset VEPs examinations in neuro-ophthalmological patients display exclusively the motion-onset VEPs pathology, we strongly recommend using them both in the theoretical vision research and ophthalmological diagnostics. Acknowledgement: Supported by the project PRVOUK - P37/07.

### • 1662

#### Pure neuroretinal dysfunction in diabetic retinopathy occurring prior to endothelial and vascular damage

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**Purpose** To search for independent neural damage in type 1 diabetic patients, in the pre-retinopathy stage of diabetic retinopathy (DR), with preserved blood-retinal barrier (BRB) permeability.

**Methods** BRB permeability was objectively measured by Vitreous Fluorometry. Neuroretinal function was assessed by standard multifocal electroretinography (mfERG) and by chromatic/achromatic contrast sensitivity (CS) (CCT-Cambridge Research Systems and Frequency Doubling Perimetry-FDT, Zeiss), in a sample of 42 patients (age=26.6±5.3years) with preserved visual acuity (VA), divided into two groups: 1. With no clinical signs of DR and normal BRB permeability (n=23eyes; VA=1.11±0.15); 2. With BRB breakdown, with no clinical signs of DR or with mild nonproliferative DR (n=61eyes; VA=1.09±0.15). These data were compared with those obtained in 25 age-matched controls (27.4±5.8years). Non-parametric statistical analysis was performed at a significance level of p<0.05.

**Results** Amplitude of neurophysiological responses was significantly decreased in all eccentricity rings in both patients groups, when compared with controls (p<0.0001). Changes in implicit time were also found in cases with preserved BRB (p<0.02). Impaired CS along the main chromatic axes was observed (p<0.03) and achromatic thresholds were also different between controls and both clinical groups (p<0.004). No correlation was found between the BRB permeability and the psychophysical and electrophysiological measurements (group with preserved BRB), confirming a lesion mechanism that is independent from endothelial/vascular changes.

**Conclusion** Retinal neuronal changes may occur in type 1 diabetes, independently of the breakdown of the BRB or onset of vasculopathy.

### • 1663

#### Ophthalmic care – Reaching out to the ageing population. Experiences in Sweden

MARTIN L

School of Health, Care and Social Welfare, Mälardalen University, Eskilstuna

**Purpose** To evaluate the suitability of a web-based inter-professional system for large-scale screening for age-related eye diseases in elderly people

**Methods** Current diagnostic devices, i.e. automatic perimeters and non-mydiatic fundus cameras were installed in ~90 optician's shops, and connected to a server. Visual acuity, refraction and intra-ocular pressure were measured and entered into the system, together with general health history and family history of eye diseases. These data sets from 30 511 screening procedures in 24 002 customers in optician's shops were evaluated by experienced ophthalmologists (n = 9), who produced a statement to the customer or, when needed, a recommendation of follow-up visit or a referral note to conventional eye care.

**Results** ICD-10 codes were recorded in 27 148 examinations. The most common code (56%) was Z01.0 Examination of eye and vision (n=15 079). Second most common diagnosis (21%) was Z71.1, i.e. suspected disease, but no diagnosis confirmed (n= 5 724). More than every fifth patient (n= 5 509 subjects) have undergone repeated screening(s). 1 760 subjects (6.5%) were referred to conventional eye care. The most common codes among these were H3, choroidal and retinal diseases (n=643) and H4, glaucoma (n=208). A survey was performed of 123 randomly selected referral responses. In 121 cases the subjects had been recommended treatment or follow-up at the clinic.

**Conclusion** The screening system, with examinations performed in optician's shops and findings evaluated by ophthalmologists, detected the expected proportion of age-related diseases with high sensitivity. The system appears to be suitable for large-scale screening of elderly people.

### • 1664

#### Characterization of human corneal grafts' transparency by optical coherence tomography and scattering measurements

HOFFART L

Marseille

**Purpose** In clinical and graft sorting applications, cornea's transparency is only subjectively qualified. The aim of this study is to bring tools to achieve transparency quantification regarding the evolution of edema within the tissue.

**Methods** The samples are human corneal grafts rejected from bank of tissue due to physiologic issues, which are submitted to swelling protocol to study their properties with edema. A multiscale analysis of the microstructure imaged by 1 μm resolved Optical Coherence Tomography (OCT) combined with a detailed characterization of backscattering properties is performed. Electromagnetic modelization is used to numerically link scattering measurements with structural defects observed with OCT.

**Results** Backscattered intensity measurements enable corneal grafts' transparency evaluation (backscattering level increases with swelling). Moreover, microstructural tissue modifications occurring during swelling (microstructure disorganization and heterogeneities) are highlighted by OCT imagery. We show that the observed heterogeneities imply higher scattering levels and explain the experimental results.

**Conclusion** Combining both techniques allows linking the scattering behavior with the evolution of microstructures within the tissue and permits to quantify corneal grafts transparency. This study has to be extended to tissues eligible for graft but this characterization in the backscattered space could directly be applied to future study of tissues before removal or to in vivo diagnosis.

• 1665

**Optic disc hamartomas in a family with heterozygous mutation in the VMD2 gene: Clinical, diagnostic and molecular genetic findings**

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(5) Kantonsspital, St. Gallen, Department of Ophthalmology, St. Gallen

**Purpose** To report a new form of juvenile-onset Best disease associated with optic disc deposits and autosomal dominant inheritance in a three-generation family.

**Methods** Five affected individuals aged from 5 to 76 years were assessed. Detailed ophthalmic examination including refraction, color fundus photographs, fundus autofluorescence imaging (FAF), optical coherence tomography (OCT), ISCEV-standard ffERG, mfERG, EOG, as well as ultrasound imaging were performed. Direct sequencing of all exons and intron-exon junction of the BEST1 gene was conducted.

**Results** The diagnosis of vitelliform maculopathy was confirmed by clinical and electrophysiological findings supported by genetic analysis. Ophthalmic imaging, OCT and ultrasound imaging showed in four out of five affected members deep subretinal deposits in the macula. Unexpected when the macula was affected, deposits located on the surface of the optic disc, showing the same echography and FAF characteristics, were observed. All five subjects had an abnormal EOG and a normal ffERG. The individual mfERG responses measured in the central, but also in the optic disc areas were reduced, the latencies were delayed. Sequencing revealed a heterozygous c.[670 C>A], coding for a L224M mutation in BEST1 in all affected subjects.

**Conclusion** Through comparison of the morphological tests: OCT, the FAF ultrasound images and the functional measure: mfERG, we deduct that the observed optic disc deposits are part of generalized disease and have the same origin as the subretinal vitelliform macular and multifocal deposits. This suggests that the described optic disc lesions are vitelliform-like optic disc hamartomas.

• 1666

**Possibility of visual prognosis based on flash visual evoked potential in cases of early brain damage**

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**Purpose** To find out whether Flash Visual Evoked Potential (FVEP) may be used for observations of visual development in cases of early brain damage.

**Methods** 94 children at the age of 5 months to 9 years were observed. Inclusion criterion was early brain damage resulting from such diseases as hypoxic-ischemic encephalopathy, hydrocephalus and various brain congenital malformations. Exclusion criteria were eye-ball diseases, retinopathy of prematurity and previous ocular surgery. The first FVEP examination was carried out at the age of 5 to 18 months, the last at the age of 5 to 9 years. FVEP was done according to ISCEV standards. The amplitude and the latency of P2 wave were measured. Children were divided into two groups: with and without visual improvement during observation. U-Mann-Whitney test was used for statistical analysis. Receiver Operating Characteristic, threshold limit value for P2 amplitude, as well as sensitivity and specificity of FVEP were calculated.

**Results** In the group of children with visual improvement P2 amplitudes were significantly higher in the second examination than in the first one (rising from mean value of 7.8 µV to mean value of 11.7 µV; p=0.0036). In the group of children with no visual improvement mean value of P2 amplitudes was 2.3 µV and P2 amplitude of 6.2 µV was threshold limit value. Vision of the most children with P2 amplitudes below this value did not improve in observation period. Sensitivity of FVEP test in visual prognosis was 94% but specificity 71.4%.

**Conclusion** P2 amplitude of FVEP is helpful in prognosis of visual development in cases of early brain damage.

**Joint Meeting: FRO - Belgian Fund for Research in Ophthalmology - part 2****• 1671**

**Identification of a novel gene for autosomal dominant retinitis pigmentosa by combined linkage analysis and whole**

*VAN CALIWENBERGH C  
Ghent*

**ABSTRACT NOT PROVIDED**

**• 1672**

**The Role of ROCK Inhibition in the Pathogenesis of Glaucoma**

*VANDE VELDES  
Leuven*

**ABSTRACT NOT PROVIDED**

**• 1673**

**The effect of aging on the regenerative potential of the zebrafish retina: insights in mammalian retinal regeneration**

*VAN HOUCKE J  
Leuven*

**ABSTRACT NOT PROVIDED**

**• 1674**

**Unraveling molecular and cellular mechanisms underlying inflammation- and aging-induced blood-retinal barrier disruption**

*VAN HOVE J  
Leuven*

**ABSTRACT NOT PROVIDED**

**• 1675**

**Histology and immunohistochemistry of the vitreolenticular interface in developmental cataracts**

VAN LOOVEREN J  
*Edegem*

**ABSTRACT NOT PROVIDED**

**• 1676**

**Functional characterization of ECSIT, a candidate disease gene for primary congenital glaucoma (PCG) on chromosome 19p13.2**

VERDIN H  
*Ghent*

**ABSTRACT NOT PROVIDED**

**• 1677**

**Self aligning recombinant human collagen scaffolds for corneal tissue engineering**

ZAKARIA N  
*Edegem*

**ABSTRACT NOT PROVIDED**

## Course 6: Retinal detachment

### • 1711

#### **Retinal detachment without PVR**

*CREUZOT C*

*Department of Ophthalmology, Dijon*

In patients suffering from a retinal detachment, the identification of all the breaks is crucial. In cases without any PVR, scleral buckling remains the first line treatment especially for ora dialysis or long standing Retinal Detachment (RD) linked to atrophic hole. However, the number, location and type of tears, the lens status will influence the selection of the treatment. The success rate is higher than 80% after one operation treated either with scleral buckling or pars plana vitrectomy but remains worse in pneumatic retinopexy. The main causes of failure are non identified retinal breaks and PVR.

### • 1712

#### **Retinal detachment with PVR**

*BERROD JP*

*Nancy*

The goal of surgery for PVR is to reattach the retina by identifying all retinal breaks and relieving all significant vitreoretinal traction. Vitrectomy, membrane peeling and gas or silicone oil tamponade are required in most cases. Firstly, a meticulous pars plana vitrectomy and shaving of the vitreous base is performed. This is followed by a 360-degree, scleral-depressed peripheral retinal examination using a wide-field viewing system to identify and then diathermize all the retinal breaks. The important goal of PVR surgery is complete membrane peeling to relieve all tangential retinal traction, thus allowing the retina to conform to the RPE/choroid. ILM peeling at the macula is also part of the treatment to prevent pucker formation or redetachment of the macula. In cases where there is persistent traction despite complete membrane peeling a sufficiently large relaxing retinotomy must be performed to relieve the persistent retinal traction. The retina is then temporarily flattened under air, or perfluorocarbon. Next, all of the retinal breaks and edges of any retinotomies are treated confluently with laser. The intraocular gas or silicone oil are typically used to provide extended long term endoretinal tamponade.

### • 1713

#### **DR in myopic patients**

*TADAYONIR*

*Paris*

#### **ABSTRACT NOT PROVIDED**

### • 1714

#### **Exudative retinal detachment**

*DE SMET MD*

*Specialized Eye Center in Uveitis and Retina, Lausanne*

Active and passive retinal water transport mechanisms must be altered to cause an exudative detachment (ED). The retinal pigment epithelium (RPE) plays a critical role. Whenever the RPE pump becomes dysfunctional or its adhesive force is overcome, ED can follow. ED can occur in the presence of reduced pump function with excessive traction as can occur diabetes, retinal detachments (mainly pseudophakic) in the absence of a tear, or certain inflammatory disorders such as pars planitis. Thickened subretinal fluid can compromise the RPE pump as is seen with persistent serous detachments following buckle surgery. However, most cases are due to a generalized depression of the RPE pump coupled with excessive fluid influx - idiopathic and steroid related central serous retinopathy, age related macular degeneration, idiopathic polypoidal vasculopathy, retinal vein occlusion, uveitis (sympathetic and VKH). Treatment will depend on the cause - may include an attempt to stimulate pump function or the elimination of tractional components. In many cases, identifying the presence of an ED leads to a conservative follow-up with excellent outcomes.

• 1715

**Tractional retinal detachment**

*MASSIN P  
Paris*

**ABSTRACT NOT PROVIDED**

## Course 7: An update on corneal infectious diseases

### • 1721

#### **Corneal bacterial infections: A practical approach**

GICQUEL JJ

Poitiers

Because of their potential to permanently impair vision or perforate the eye, bacterial corneal ulcers are an ophthalmologic emergency. They usually follow an insult (sometimes minor) in the corneal epithelium that provides an entry for bacteria. The increased use of soft contact lenses in recent years has led to an important rise in the occurrence of bacterial ulcers (especially aggressive Gram- bacteria). The goal of this course is to present a practical, systematic approach of the management of bacterial corneal ulcers, as well as an update on new concepts in the diagnosis and treatment of those conditions.

### • 1722

#### **Herpes and Zoster infections update**

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(2) Laboratoire de Virologie Moléculaire et Structurale, 91198 Gif sur Yvette

Herpes Simplex Virus (HSV) and Varicella-zoster Virus (VZV) are the two leading causes of infectious acquired amblyopia. After a phase a latent infection in the trigeminal ganglia, they may reactivate to eventually infect the cornea. There are several common features of keratitis due to HSV or VZV. The most frequent forms involve the epithelium, with 4 different patterns: punctuate and diffuse, dendritic, geographic, and marginal. Stromal forms of keratitis are mostly non-necrotic, and usually resolve with a combination of oral antivirals and topical steroids. In contrast, a necrotic stromal keratitis usually requires intravenous antiviral therapy. Endothelial forms of HSV/VZV keratitis are far less frequent. Three different types may be observed: disciform, diffuse or linear, the latter one being the most aggressive. After the acute phase of HSV/VZV keratitis, preventive treatment with oral antivirals may be given according to the clinical history. For VZV, a vaccine is now available. Its effectiveness has been shown for the prevention of herpes zoster ophthalmicus, but its place in patients with recurrent VZV infections of the eye remains to be clarified.

### • 1723

#### **The particularities of corneal infectious diseases in children**

BREMONT-GIGNAC D

Amiens

Ocular surface bacterial infections affect subjects of all ages with a high frequency in newborns and children. In infant, children and teenagers, the most common ocular pathogens, which differ from adults, are *Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and also *Moraxella* species. These infections could lead to ulcers and sight-threatening complications. Corneal infections are not common but can be difficult to diagnose because the pathology can develop without pain. In addition the examination may be difficult to perform. The treatment should be provided earlier and aims to eliminate the bacteria, virus or fungal pathogens. The specific epidemiology of pathogens will be detailed according to the age. Risks of visual impairment and amblyopia must be integrated. An update on topical antibiotics and current options will be reviewed with practical aspects, diverse clinical cases and considering quality of life of the children and parents.

### • 1724

#### **New emerging treatments in severe corneal infectious diseases**

GICQUEL J (1), DUA HS (2)

(1) Poitiers

(2) Nottingham

#### **ABSTRACT NOT PROVIDED**

## SIS: New frontier in glaucoma surgery: Trans-trabecular meshwork

### • 1731

#### iStent implant

*AUL*

*Manchester Royal Eye Hospital, Manchester*

istent is a novel device that canulates Schlemm's canal to enhance physiological outflow and lowers intraocular pressure. Results of istent surgery combined with phaco would be presented, together with the results of intraoperative flow test. Results of multiple istents and istent Supra would also be presented. New device like the Hydrus would also be demonstrated.

*Commercial interest*

### • 1732

#### Trabectome surgery

*LONGSTAFF*

*Sheffield*

**ABSTRACT NOT PROVIDED**

### • 1733

#### Other trans-trabecular meshwork implants

*LIM K (1, 2)*

*(1) London*

*(2) Ophthalmology, St Thomas' Hospital, London*

This lecture will review new generation of Trans-TM devices that are currently in trial or development.

*Commercial interest*

### • 1734

#### Basic science behind trabecular meshwork surgeries

*OVERBY D*

*Imperial College London, Dept. of Bioengineering, London*

Trans-trabecular meshwork surgeries attempt to lower intraocular pressure (IOP) by shunting across the trabecular meshwork (TM) that is believed to generate the bulk of aqueous humour outflow resistance. Despite elevated TM resistance in glaucoma, newer trans-TM surgeries appear to be less successful at lowering IOP compared to traditional filtration surgeries (Mosaed et al. Trans. Am. Ophthalmol. Soc., 2009). To understand the mechanism and possible limitations of trans-TM surgeries, we review the anatomy and basic science of outflow resistance generation within the TM and post-TM aqueous vessels. These studies provide estimates of IOP expected from trans-TM shunts and reveal obstacles that may limit the efficacy of trans-TM surgeries.

• 1741

**Rod microglia is absent from microglial changes in mice retina contralateral to experimental glaucoma**

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**Purpose** The aim of the present study was to analyze the presence of rod microglia in a model of unilateral laser-induced ocular hypertension (OHT).

**Methods** Adult albino Swiss mice were divided into two groups: naïve (age-matched control; n=6) and laserized (n= 6). Retinal whole-mounts were analyzed by immunostaining using antibodies against Iba1, MHC-II, ED1 and NF-200.

**Results** The nerve-fiber layer (NFL) of contralateral and OHT-eyes had ramified microglia related to blood vessels, similar to those of aged-matched control eyes. However, only OHT-eyes had rod microglia. Rod microglia lay parallel to and close to the axons and were related to retinal ganglion cells (RGCs) showing signs of degeneration (NF-200+RGCs). In some instances, rod microglia processes surrounded the soma and proximal dendrites of NF-200+RGCs and penetrated the underlying RGC layer and inner plexiform layer. Microglia had morphological signs of activation and MHC-II expression up-regulation in both, the contralateral and OHT-eyes. However, only in OHT-eyes did rod microglia show ED1 staining throughout the NFL. With respect both to morphological features and to ED1 immunostaining patterns, three stages of rod microglia were detected, with processes shortening, somas thickening, and ED1 immunostaining increasing progressively from stage 1 to 3.

**Conclusion** After 15 days of unilateral laser-induced OHT, rod microglia was restricted to eyes with OHT and degenerated NF-200+RGCs but absent in contralateral eyes. The microglia activation observed in contralateral eyes could be related with neuroprotection.

• 1742

**Neuropeptide Y Y1 receptor is neuroprotective and modulates microglia reactivity in the rat retina**

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**Purpose** Neuropeptide Y (NPY) is neuroprotective and can regulate inflammation. In the retina, microglia (MG) activation has been implicated in the pathogenesis of several diseases. We investigated whether NPY, particularly through Y1 receptor (Y1r) activation, is neuroprotective and modulates pro-inflammatory responses in the retina.

**Methods** Immunocytochemistry, RT-PCR, TUNEL and ELISA were used.

**Results** NPY and NPY Y1, Y2 and Y5 receptors were detected in retinal ganglion cells (RGC) and MG. The increase in TUNEL-positive cells in the ganglion cell layer induced by exposure of retinal explants to NMDA was inhibited by Y1r activation. Exposure to LPS activated retinal MG, and NPY prevented MG morphological changes via Y1r. NPY or [Leu31, Pro34]-NPY (LP-NPY; Y1r/Y5r agonist) inhibited the increase in iNOS immunoreactivity triggered by LPS, and BIBP 3226 (Y1r antagonist) abolished the effect of LP-NPY. LPS also increased the concentration of TNF-alpha, IL-1beta and IL-6. NPY inhibited the increase in IL-1beta and IL-6, but not TNF-alpha. Moreover, LP-NPY inhibited the increase in TNF-alpha, IL-1beta and IL-6, and BIBP 3226 abrogated the effect of LP-NPY.

**Conclusion** In conclusion, RGC and MG express NPY and NPY Y1, Y2 and Y5 receptors. The activation of Y1r can exert neuroprotective effects in RGC and induce anti-inflammatory effects in the retina, inhibiting MG activation. Support: FCT (PTDC/SAU-NEU/099075/2008, PTDC/NEU-OSD/1113/2012, PEst-C/SAU/UI3282/2011-2012, PEst-C/SAU/LA0001/2011-2012, SFRH/BPD/69123/2010, SFRH/BD/44817/2008), Portugal, and COMPETE-FEDER.

• 1743

**Effect of neuroprotective statins in retina: A hypercholesterolemic rabbit model**

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**Purpose** The aim of the present study was to analyze qualitative and quantitative retinal changes in hypercholesterolemic rabbits after a low-dosage statin treatment and to evaluate their possible neuroprotective effect on the retina.

**Methods** New Zealand rabbits were divided into three groups: Control (G0; n=6), fed a standard diet; Hypercholesterolemic (G1, n=6), fed a 0.5% cholesterol-enriched diet for 8 months; and Statins (G2; n=6), fed a 0.5% cholesterol-enriched diet for 8 months plus administration of fluvastatin sodium or pravastatin sodium at a dosage of 2 mg/Kg/day each. Eyes were processed and analyzed for transmission-electron microscopy. Semi-thin sections were used to quantify cell number and thickness of retinal layers (excluding retinal pigment epithelium and nerve-fiber layer) under light microscopy.

**Results** G1 and G2, in comparison to G0, had a decreased cell number in the outer nuclear layer (ONL) ( $p<0.05$  and  $p<0.005$ , respectively). No differences were detected in the inner nuclear layer or ganglion-cell layer among the study groups. In G2, the ultrastructural cell morphology was similar to G0 and there were fewer signs of necrosis and apoptosis than in G1. Although the ONL cell number was decreased in G1, the retinal thickness in this group showed no differences in comparison to G0 due to retinal edema. Retinal thickness in G2 was reduced with respect to G1 ( $p<0.05$ ) because of the absence of retinal edema, and to G0 ( $p<0.05$ ) due to ONL cell loss.

**Conclusion** Low-dosage statins did not prevent ONL cell loss but preserved the normal ultrastructure of the remaining retinal neurons and avoided hypercholesterolemia-induced retinal edema.

• 1744

**Modified connexin43 mimetic peptide shows higher efficacy in reducing retinal ganglion cell loss and vessel leak after retinal ischemia**

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(2) School of Chemistry and Molecular Biosciences and School of Pharmacy, University of Queensland, Brisbane

**Purpose** Optic neuropathy is associated with retinal ganglion cell (RGC) loss leading to optic nerve damage and visual impairment. Transient block of connexin43 (Cx43) hemichannels by mimetic peptides (MP) after retinal ischemia has been shown to inhibit uncontrolled hemichannel opening and may provide improved RGC survival. However, high hydrophilicity and poor peptide stability can limit efficient delivery in a clinical setting. This study evaluated the efficacy of modified Cx43MP using a retinal ischemia reperfusion rat model.

**Methods** Retinal ischemia was created in the left eye (120 mmHg for 60 min) with the right eye serving as a control and Cx43MP formulations were injected intravitreally. Evan's blue dye was injected intraperitoneally 4 h after reperfusion to visualize vessel leak using confocal microscopy. Retinal whole mounts were also labeled with Cx43, GFAP and Brn3a antibodies to quantify Cx43 and RGC densities.

**Results** Intravitreal injection of native and modified Cx43MP resulted in significantly reduced Cx43 levels compared to untreated eyes 8 h after ischemia, with Cx43MP containing two C12-lipoamino acid groups reducing Cx43 counts almost down to baseline level. A similar trend was seen for RCG counts with the modified Cx43MP resulting in >93% RCG survival after 28 days compared to <70% for untreated eyes. Vessel leak was also noticeably reduced in Cx43MP treated eyes.

**Conclusion** C12-lipoamino acid conjugation to Cx43MP increased peptide stability and may have also resulted in better tissue permeability, improving the overall efficacy of Cx43MP. Modified Cx43MP may therefore offer a clinically relevant treatment option for optic neuropathies.

• 1745

**Effect of breathing 12% oxygen in nitrogen on retinal blood flow and oxygen saturation**

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**Purpose** It has been reported that altering the fraction of oxygen in inhaled air changes retinal arterial and venous oxygen saturation. In the present study we set out to study the effects of 12% oxygen in nitrogen breathing on retinal blood flow and retinal oxygen saturation.

**Methods** 30 healthy volunteers were included into the present study. Assessment of the effect of 12% oxygen in nitrogen breathing on retinal blood velocities was performed using laser Doppler velocimetry (LDV). Retinal vessel diameters were assessed with the Dynamic Vessel Analyzer (DVA, Imedos, Germany). Retinal oxygen saturation was investigated using the same instrument based on spectroscopic evaluation of the data. The effects of 12% oxygen in nitrogen breathing on systemic oxygen tension was evaluated based on arterialized blood samples drawn from the earlobe.

**Results** Breathing 12% oxygen in nitrogen caused systemic hypoxia as evidenced from the reduced systemic oxygen tension ( $p < 0.01$ ). In addition, a reduction in both arterial and venous oxygen saturation was seen in retinal vessels ( $p < 0.01$  each), which was more pronounced in veins. In parallel we observed an increase in retinal vessel diameters, retinal blood velocities as well as in retinal blood flow ( $p < 0.05$  each).

**Conclusion** The results of the present study indicate good validity of retinal oxygen saturation measurements using the DVA. In addition, the results are compatible with unchanged retinal oxygen extraction during hypoxia.

• 1746

**Optic nerve head autoregulation during changes in arterial blood pressure**

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**Purpose** Previous studies in our laboratory have shown that an increase in blood pressure during isometric exercise leads to an increase in optic nerve head blood flow (ONHBF). An interesting phenomenon was found in some subjects when they were exhausted from isometric exercise and blood pressure decreased slightly. These periods were sometimes associated with a pronounced decrease in ONHBF even though ocular perfusion pressure (OPP) was still much higher than at baseline. The present study aimed to investigate this phenomenon in more detail and to gain insight in the regulatory mechanisms of ONHBF during short-term fluctuations in OPP.

**Methods** 40 healthy subjects aged between 18 and 35 years were included in this study. Three periods of baseline measurements at rest and 3 periods of measurements during isometric exercise with a handgrip were alternated while ONHBF was measured continuously using laser Doppler flowmetry. Mean arterial blood pressure (MAP) was measured every minute and OPP was calculated as 2/3 MAP – intraocular pressure (IOP).

**Results** OPP significantly increased during handgripping compared to the resting periods ( $p < 0.001$ ), whereas ONHBF was not significantly altered during these changes in OPP indicating for autoregulation. Three subjects showed a decrease of more than 10% in ONHBF during recovery at all 3 periods of isometric exercise. Unexpectedly, however, 3 other subjects consistently showed an ONHBF reduction of more than 10% during isometric exercise.

**Conclusion** The present study indicates a complex regulation of ONHBF. Six subjects (15%) showed an abnormal ONHBF response during handgripping. This is most likely caused by an exaggerated vasoconstrictor response in order to counteract the increase in OPP.

## SIS: How biologic agents are changing the story of uveitis: anti-TNF- $\alpha$ agents and more

• 1751

### The evolution of monoclonal antibodies

DICK A

*School of Cellular and Molecular Medicine, Bristol*

Since the Milstein's explosive generation experimentally of monoclonal antibodies both the ability to probe experimentally and deliver therapeutically has created a wave of utilising 'biologics' for therapeutic gain. From understanding of inflammatory responses in the eye during uveitis we have been able to iteratively target either cytokine responses, cell responses or inhibit activation of cells. The introduction will give a brief overview of developments, targets and the future in a more specified and personalised approach to harnessing the strengths of biologic therapy.

• 1752

### What I should do before considering biologic agents

WILLERMAIN F

*Bruxelles*

By definition, a biologic agent is a molecule produced by a living organism but chemically synthesized or modified product such as peptides or nucleotides are also sometimes included. Biological agents usually referred to molecules that precisely interact with crucial pathways involved in disease development. In the context of non infectious uveitis, and with the important exception of interferons, biological agents are classically highly specific immunosuppressive agents. Some of them have been associated with tremendous clinical effects in severe non infectious uveitis patient. A common problem with their use is related to serious primary infection or reactivation of latent infectious diseases such as tuberculosis. The issue of worsening or inducing MS in intermediate uveitis in the case of anti-TNF is also important as well as their high cost. They are thus usually proposed to patients resistant to conventional immunosuppressive treatment, after having eliminated infectious causes and latent systemic infection. However, their use as first line agent in certain uveitis conditions is often debated. In this SIS, we will review all those important general questions related to the use of biological agents in non infectious uveitis.

• 1753

### Long-term clinical outcomes in patients with refractory uveitis associated with Behcet's disease treated with infliximab

ABU EL ASRAR A

*Department of Ophthalmology, Riyadh*

Purpose: To assess long-term efficacy and safety of infliximab for refractory Behcet's disease (BD) uveitis and to evaluate the effect of withdrawal of infliximab after achieving long-term remission. Methods: Retrospective study of 19 patients. Results: Mean follow-up was  $44.1 \pm 36.5$  months and mean number of infliximab infusions was  $21.6 \pm 14.6$ . At end of follow-up, there was significant improvement of visual acuity and reduction of central macular thickness. All patients achieved remission, 14 of whom were able to discontinue corticosteroids. Ten patients developed autoantibodies and one patient developed infusion reactions. Eight eyes underwent intraocular surgery without exacerbation of quiescent uveitis. After achieving complete remission, 5 patients discontinued infliximab and maintained remission during a mean of  $24.6 \pm 5.5$  months. Conclusions: Infliximab is effective and safe for long-term treatment for refractory BD uveitis. Repeated infusions are required to maintain long-term remission which may be sustained despite withdrawal of infliximab. Induction of autoantibodies is common.

• 1754

### Rescue therapy with subcutaneous anti-TNF- $\alpha$ agents for idiopathic non-infectious uveitis

NERI P (1), ARAPI I (2), PIRANI V (2), CAPIANO V (2), GIOVANNINI A (2), MARIOTTI C (2)

(1) Agugliano

(2) *The Eye Clinic, Polytechnic University of Marche, Ancona*

Chronic non-infectious uveitis (NU) remains one of the most challenging problems in ophthalmology. Often, early and aggressive treatment is needed for a good visual acuity outcome. Local and oral corticosteroids remain the first line of treatment, even though side effects, such as glaucoma, cataract and Cushing Syndrome, can be serious and not tolerable. Therefore, a variety of immunosuppressive agents are used. Recently, new types of drugs, called "biologic agents," are available. In the model of experimental autoimmune uveitis (EAU) it has been demonstrated that tumor necrosis factor-alpha (TNF- $\alpha$ ) may play a key role in uveitis. Beside the well known intravenous anti-TNF- $\alpha$  agent called infliximab, other subcutaneous anti-TNF- $\alpha$  drugs have been introduced, such as Adalimumab and Golimumab. Adalimumab presents a stronger medical literature and it seems to present promising qualities. Few reports are available for Golimumab in uveitis at this time. Adalimumab can control uveitis unresponsive to the traditional immunosuppressive agents and, moreover, its subcutaneous administration makes the procedure user friendly. This drug can be used in selected cases as a rescue therapy for idiopathic non-infectious uveitis.

**• 1755****The “strange story” of Interferon- $\alpha$ : A very peculiar biologic agent**

BODAGHIB  
Paris

Biologic agents are widely used in patients with uveitis. Interferon alpha is one of the first agents tested in this indication. Its efficacy was reported in the early 90's in patients with severe and refractory Behçet's disease-associated uveitis. Several other studies from different centers have confirmed the initial results. However, this agent is mostly used in Europe. Severe posterior, intermediate and panuveitis may respond to interferon alpha. Some infectious entities such as herpetic viral retinopathies are also appropriate candidates. Doses vary between centres and countries. Tolerance is a major factor to consider. The drug may be discontinued in up to 40% of cases for various side effects. Interferon alpha is a double-edged sword in a few situations. Long-term remission or healing may be obtained after long-term use of the drug.

## Course 8: Visual electrophysiology

### • 1761

#### A general introduction to the tests and abnormality localisation

LEROY B  
Ghent

Purpose: To illustrate which electrophysiological tests are available to the visual electrophysiologist, and to highlight when and where they can be used to learn more about the patient's condition. Methods: A systematic overview of the different electrophysiological tests will be given, including a physiological and anatomical correlation for each test. Results: Each of the different electrophysiological tests available allows the clinician to evaluate the function of different aspects of the retina and/or optic nerve. In so doing, essential information is obtained in order to pinpoint the exact location of the dysfunction, and make a correct diagnosis. Conclusions: Visual electrophysiology is essential to make a correct diagnosis both for acquired and inherited retinal and optic nerve diseases.

### • 1762

#### Electrophysiology in acquired disease

HOLDER G  
Moorfields Eye Hospital, London

This presentation will address the use of electrophysiology in various acquired disorders, including vascular, nutritional, toxic, inflammatory, parasitic, autoimmune and paraneoplastic disease. A case-based format will be used to demonstrate the contributions of electrophysiology both to diagnosis and management.

### • 1763

#### Electrophysiology in inherited disease

LEROY B  
Ghent

Purpose: To illustrate the added value of visual electrophysiology in the diagnosis of inherited retinal dystrophies and dysfunctions, and their management in the ophthalmic genetic clinic. Methods: A systematic overview with a case presentation format will be used to illustrate different genetically determined retinal dystrophies and dysfunctions. Case descriptions will include clinical and electrophysiological phenotypes as well as genotypes. Results: Phenotypes and genotypes of genetically determined retinal diseases are very different, and visual electrophysiology is essential in making a correct diagnosis. Conclusions: Taking a thorough history in combination with an extensive clinical examination and psychophysical and electrophysiological tests most often allows a to make a specific diagnosis when dealing with inherited retinal disease. Visual electrophysiology also allows to distinguish between progressive and stationary conditions, and it provides essential information regarding evolution of progressive disease.

### • 1764

#### Electrophysiology and imaging

HOLDER G  
Moorfields Eye Hospital, London

There have been major advances in retinal imaging techniques in recent years, with both fundus autofluorescence imaging and spectral-domain optical coherence tomography adopting increasingly important roles in management and diagnosis. This presentation will utilise a case-based format address the relationship between structure and function in various acquired and inherited disorders.

## • 1771

**Effect of hypoxic stress on induction and plasticity of tumour-induced immune cells in uveal melanoma**

BRONKHORST IHG (1), JEHS TML (1), DIJKGRAAF EM (2), LUYTEN GPM (1),  
VAN DER VELDEN PA (1), VAN DER BURG SH (2), JAGER MJ (1)  
(1) Ophthalmology LUMC, Leiden  
(2) Clinical Oncology LUMC, Leiden

**Purpose** Highly malignant uveal melanoma contain increased numbers of lymphocytes and macrophages. We wondered whether hypoxia plays a role in the development of this inflammation. We analysed whether hypoxia induces uveal melanoma cells to release pro-inflammatory cytokines, and whether tumor supernatant (TSN) affects monocyte migration and differentiation.

**Methods** The expression of pro-inflammatory genes in freshly cultured uveal melanoma was studied in an *in vitro* 24 hour hypoxic culture system using qPCR. Cell lines were cultured under normoxic and hypoxic conditions. Chemotaxis was tested using a transwell system with purified monocytes and TSN. Differentiation was tested by adding TSN to a monocyte-DC culture. CCL2, IL-6 and PGE2 levels in TSN were determined by ELISA.

**Results** Exposure of freshly-cultured uveal melanoma cells to hypoxia led to an increased expression of the pro-inflammatory cytokines PLGF, TGF $\beta$ , END1, ICAM1 and a lower expression of AIM1 (EMAP2), CCL2 (MCP-1), and IL1b. TSN from melanoma cell lines, cultured in normoxic as well as hypoxic conditions, was able to attract monocytes. Migration was independent of tumor-produced CCL2. Uveal melanoma supernatant inhibits monocyte differentiation.

**Conclusion** Under hypoxic conditions, immune response genes are differentially expressed in cultured primary uveal melanoma cells. TSN from uveal melanoma cell lines is capable of affecting the chemotactic response of monocytes *in vitro*, irrespectively of the hypoxic or normoxic conditions. Our data suggest that the induction of immune cells is not dependent on intratumoral oxygen level, and the uveal melanoma TSN does not skew macrophage polarization phenotype.

## • 1772

**Targeting the hypoxia pathway in uveal melanoma cells**

ASNAGHIL (1), LIN MH (1), LIM KS (1), LIM KJ (1), BAR EE (2),  
EBERHART CG (1, 3)  
(1) Department of Pathology, Johns Hopkins University, School of Medicine, Baltimore  
(2) Department of Neurological Surgery, School of Medicine, Case Western Reserve University, Cleveland  
(3) Department of Ophthalmology, Wilmer Eye Institute, Johns Hopkins University, School of Medicine, Baltimore

Uveal melanoma is the most frequent intraocular cancer in adults and cause significant mortality due to hematogenous dissemination. Hypoxia-inducible factor 1 $\alpha$  (HIF1 $\alpha$ ), a master regulator of the hypoxic response, is associated with aggressive uveal melanomas. Here we investigated the mechanism responsible for the prometastatic effect of HIF1 $\alpha$  and its downstream targets. HIF1 $\alpha$  protein was relatively abundant in the OCM1, OMM1, Mel290 and 92.1 lines grown in normoxia (21% pO<sub>2</sub>), and was further induced in hypoxia (1% pO<sub>2</sub>), as well as the mRNA levels of VEGF and LOX, two transcriptional targets of HIF1 $\alpha$ . Interestingly, low oxygen tension increased the ability of uveal melanoma cells to invade Matrigel. Genetic suppression of HIF1 $\alpha$  by shRNA significantly decreased growth in hypoxia ( $p<0.0001$ ) and impaired cellular invasion both in normoxia and hypoxia ( $p<0.0001$ ), suggesting that HIF1 $\alpha$  is necessary for invasion even in normoxia. To determine downstream targets, we examined Notch pathway members, which we have previously linked to uveal melanoma invasion, and found that Notch ligand Jag2 and target Hey1 were induced by hypoxia, and their expression was reduced using HIF1 $\alpha$  shRNA, suggesting a crosstalk between hypoxia and Notch pathways.

## • 1773

**Epigenetic regulation of the epithelial phenotype in uveal melanoma**

VERSLUIS M, DE LANGE MJ, VAN PEELTS, LUYTEN GPM, JAGER MJ,  
VAN DER VELDEN PA  
Ophthalmology, Leiden

**Purpose** The presence of epithelial cells is a bad prognostic factor in uveal melanoma (UM) and epithelial tumors are characterized by high expression of E-cadherin. However, CDH1, the gene encoding for E-cadherin, is located at chromosome 16q which is lost in UM with a bad prognosis. In order to solve this paradox we studied the underlying epigenetic mechanism of the spindle to epithelial transition and the accompanying E-cadherin expression.

**Methods** UM cell lines were *in vitro* treated with methylation and deacetylation inhibitors, or were cultured under hypoxic conditions to mimic *in vivo* UM conditions to induce epigenetic reprogramming. RNA and DNA was isolated for gene expression, DNA methylation, and chromosome structure analysis, while cell lysates were used for E-cadherin analysis.

**Results** Treatment with methylation and deacetylation inhibitors resulted in a phenotypic change from a spindle to more epithelial cell type. Furthermore, inhibition of methylation and hypoxia treatment both upregulated E-cadherin expression *in vitro* and induced euchromatin formation at 16q22, where CDH1 is located.

**Conclusion** By revealing epigenetic regulation of CDH1 in UM we resolve a step in the dynamic process of spindle to epithelioid celltype switch in UM. Since this switch could be induced by hypoxia we establish a link between environmental stress and progression. With these data we provide evidence for epigenetic regulation of UM progression as well as an option for future treatment.

## • 1774

**Gain of chromosome 6 status does not influence HLA-expression in uveal melanoma**

VAN PEELTS (1), VAN ESSEN TH (1), BRONKHORST IHG (1), VERSLUIS M (1),  
LUYTEN GPM (1), VAN HALL T (2), VAN DEN ELSEN PJ (3),  
VAN VELDEN PA (1), JAGER MJ (1)  
(1) Ophthalmology, Leiden  
(2) Clinical Oncology, Leiden  
(3) Pathology, department of Immunohematology and Blood Transfusion, Leiden

**Purpose** Chromosomal aberrations and the inflammatory phenotype have been identified as predictive factors for survival of uveal melanoma (UM). The mechanism by which these factors are linked remains obscure. We took the human leukocyte antigen (HLA), located on chromosome 6p as marker for inflammation and studied whether aberrations of chromosome 6p influenced the HLA expression in UM.

**Methods** SNP-array copy number analysis and gene expression profiling were performed on 28 uveal melanomas of patients who underwent enucleation between 1999 and 2004 at the Leiden University Medical Center, Leiden, in The Netherlands. UM protein expression of HLA-A, -B and -DR was measured with immunohistochemistry. The status of chromosome 3, 6p, and gene expression of HLA and HLA regulators were analyzed, as well as protein expression of HLA.

**Results** Gain of 6p was present in 8 cases (29%) and not related with survival. An increased gene expression was seen in the group who died due to metastatic UM for HLA-A and -B, and B2M. Gene expression correlated with protein expression for HLA-A, HLA-B, but not with HLA-DR. Tumors did not differ in level of HLA protein expression, HLA gene expression, and HLA regulator gene expression with regard to chromosome 6p status.

**Conclusion** High HLA gene- and protein expression in UM are not influenced by gain of 6p. Yet for the first time we demonstrated that in UM an increased expression of HLA class I genes correlated with the elevated protein expression of HLA class I.

## Free papers P0 1/2: New insights into uveal melanoma

### • 1775

#### A new and standardized method to sample and analyse vitreous biopsies by the Cellient® automated cell block system

VAN GINDERDEUREN R (1, 2), VAN CALSTER J (1)

(1) *Ophthalmology, Leuven*

(2) *Pathology, Leuven*

**Purpose** In this prospective study a universal protocol for sampling and analysing vitreous material was investigated. Vitreous biopsies are difficult to handle because of the paucity of cells and the gelatinous structure of the vitreous. Histopathological analysis of the vitreous is useful in difficult uveitis cases to differentiate uveitis from lymphoma or infection and to define the type of cellular reaction

**Methods** 170 consecutive vitreous samples were analysed with the Cellient® tissue processor (Hologic). This machine is a fully automated processor starting from a specified container with PreservCyt® (fixative fluid) with cells to paraffin. Cytology was compared with fixatives Cytolyt® (contains a mucolyticum) and Preservcyt®. Routine histochemical and immunostainings were evaluated

**Results** In 92% of the cases, sufficient material was found for diagnosis. In 14%, a Cytolyt® wash was necessary to prevent clotting of the tubes in the Cellient® due to the viscosity of the sample. In 23% the diagnosis was an acute inflammation (presence of granulocytes), in 33% chronic active inflammation (presence of T-lymphocytes), in 33% low-grade inflammation (presence of CD68 cells, without T-lymphocytes); and in 3% a malignant process

**Conclusion** A standardized protocol for sampling and handling vitreous biopsies, fixing in PreservCyt® and processing by the Cellient® gives a superior result in morphology, number of cells, and possibility of immuno-histochemical stainings. The diagnosis can be established or confirmed in more than 90% of cases

### • 1776

#### Ophthalmic histopathology samples – are we sending enough?

DEV BORMAN A (1), HASSANI M (2), KESHARAJI R (1), NADERIK (3), GHAZI-NOURIS (1)

(1) *Ophthalmology, Mid Essex Hospital Services NHS Trust, Chelmsford*

(2) *Peninsula College of Medicine and Dentistry, Plymouth*

(3) *Colchester Hospital University NHS Foundation Trust, Colchester*

**Purpose** To ascertain the range of histopathological diagnoses made from tissues removed during ophthalmic surgical procedures over two years from one NHS Trust. During this period, joint recommendations were published by the Royal College of Pathologists (RCPATH) and the Royal College of Ophthalmologists (RCOphth) in the United Kingdom, regarding the referral of ophthalmic pathology specimens, stating that in order to avoid delayed or missed diagnoses, with certain exceptions, all tissues obtained from ophthalmic surgical procedures should be sent for histopathological examination. This policy was implemented locally from January 2011.

**Methods** This was a retrospective case note review of the ophthalmic histopathology specimens sent between January 2010 and December 2011, at the Mid Essex Hospital Services NHS Trust. Patient demographics and histopathological diagnoses were determined for each.

**Results** Over the two-year period, 268 specimens were sent for ophthalmic histopathological examination. In 2010, 112 samples were sent, with 18 malignancies detected (16.1%). In 2011, 156 samples were sent, with 30 malignancies identified (19.2%). The most common diagnosis was basal cell carcinoma, comprising 43/48 malignancies (89.6%).

**Conclusion** Since the local implementation of the joint guidelines from the RCPATH and RCOphth, there was an increase in the number of ophthalmic histopathology specimens sent within this Trust, with a 3.1% increase in the number of malignancies detected. We believe that this improvement in detection of ocular malignancies justifies the increased burden on the histopathology service from the ophthalmology unit within this Trust.

### • 1777 / S089

#### Orbital melanocytosis and OTA naevus

DE KEIZER R JW, LAUWERS N, DE GROOT V

*Ophthalmology, Antwerp*

**Purpose** Presentation and discussion of a rarely reported location of orbital melanocytosis

**Methods** Case report

**Results** In a 54 year old man with an oculodermal melanocytosis (naevus of OTA), enucleation was performed for a choroidal melanoma. During the surgery spots of hyperpigmentation, based on melanocytosis, were found in tendons, muscles and orbital fat. Oculodermal melanocytosis is classically described on the periocular skin, sclera, uvea, orbit, meninges, palate or tympanic membrane and is a well-known risk factor to develop choroidal and orbital melanoma. Few reports describe the particular involvement of the orbital tissue. We show the pictures of orbital tissue and extraocular muscle pigmentation and explain it with embryology.

**Conclusion** Even after enucleation for a choroidal melanoma in an OTA naevus, the ophthalmologist should be alert that extraocular melanocytes can remain and potentially be the cause of an orbital melanoma.



**THURSDAY  
SEPTEMBER 19  
2013**

**• 2211****Pathophysiology of macular edema**

*JONAS J  
Mannheim*

The talk will present and discuss causes and treatment possibilities of macular edema in various retinal diseases.

*Commercial interest*

**• 2212****Can we quantify macular edema outcome?**

*MASSIN P  
Paris*

**ABSTRACT NOT PROVIDED**

**• 2213****Functional assessment of macular edema**

*MIDENA E (1, 2)*

(1) *Department of Ophthalmology, University of Padova, Padova*  
(2) *GB Bietti Foundation, Roma*

**Aim** Macular edema is evaluated using pure morphologic imaging tests and visual acuity as sole visual function test. Unfortunately, visual acuity just partly reflects visual function. A new integrated morpho-functional approach seems useful.  
**Methods** Patients affected by macular edema were investigated using a multimodal/integrated imaging approach including currently available pure morphological retinal imaging techniques and microperimetry. Results The correlation between morphologic and functional imaging modalities strictly depends on the different involvement of retinal layers by the pathologic process. Data shows that visual function impairment is better quantified when microperimetry is added to the currently available diagnostic armamentarium.  
**Conclusions** Both in clinical trials and in daily clinical practice visual function needs to be better investigated to cope with patient's requests. Adding reliable, topographically related visual functional data, currently obtained by microperimetry, to other pure retinal imaging investigations allows not only to better identify different "clinical phenotypes" of macular disorders, but also to provide invaluable information about treatment and retreatment criteria.

**• 2214****How to select our treatments?**

*CREUZOT C  
Department of Ophthalmology, Dijon*

The analysis of associated circumstances to macular edema will help the clinician to select one among the different therapeutic options of macular edema (ME). The duration of ME, the efficacy of previous treatments, the ability of the patient to be examined regularly will influence this choice. The different options are grid laser, antiVEGF agents, steroids. Associated risk factors like severe glaucoma or lens status also need to be considered. The control of systemic conditions remain a key issue. The strategy can change during the follow-up according to the efficacy and the recurrences observed with the treatments.

*Commercial interest*

**SIS: Macular edema****• 2215****How to organise the follow-up of patients with macular edema?***POURNARAS CJ**Ophthalmology Group, Rothschild Memorial, Genève*

The results of clinical trials have demonstrated significant visual and anatomic benefits to intravitreal agents in the treatment of the macular edema (MO) related to retinal ischemic microangiopathies. Following the evaluation of non-perfused capillary areas, on fluorescein angiography, spectral domain optical coherence tomography (OCT) helps to quantify the amount of cystoid macular edema. The clinical examination include VA assessment, biomicroscopy and OCT. Important differences exist in the protocols evaluating treatment for Diabetic MO, from the mandatory monthly anti VEGF injections, to either protocol, requiring injections during the first 4 study visits and prompt or deferral laser or in trials, where patients, after 3 mandatory monthly injections, had both a variable dose and injection, based on VA and OCT evaluation. The MO related to RVO should be monitored monthly for the first 3 months, and then every 2 months for the first year. During this monitoring period, patients should be instructed to return promptly whenever they notice a decrease in vision, a possible indication of macular edema, or the conversion to ischemia. Follow-up after the initial 6 months will be required.

• 2221

**Definition and diagnosis of advanced glaucoma**

ZEYEN T

*Leuven*

According to Hodapp's criteria for visual field (VF) scoring, advanced glaucoma can be defined as having a VF mean deviation index (MD) < -12 dB. Advanced glaucoma can also be defined on the basis of the patient's skills to perform daily activities. Functional tests are usually more useful than structural parameters to follow-up patients with advanced glaucoma. An overview of the available investigative techniques in advanced glaucoma will be provided.

• 2222

**Treatment of advanced glaucoma**

BRONA

*Dijon*

Glaucoma is still the second cause of irreversible blindness worldwide. One of the main reasons is that since glaucoma is a symptom-free disease, at least 50% of glaucoma patients are not aware of their disease. Therefore it is not uncommon to see at the consultation advanced glaucoma cases even in the Western world. The management of these advanced cases is very challenging because if at best the treatment can stop the loss of retinal ganglion cells (RGCs) while lowering intraocular pressure (IOP), it will not prevent from the natural age-related loss of RGCs. Therefore many of these patients will go blind even with a "successful" treatment. The modalities of the treatment are controversial because when surgery is indicated, the fear of the so-called whipe-out syndrome is well known among glaucoma surgeons. Recent papers have highlighted that this complication may occur but may be at a lower rate than previously thought. Fair information given to the patients by the ophthalmologist is probably at least as important as the treatments

• 2223

**Differential diagnosis**

ANSARIE

*Maidstone*

There are many conditions that can mimic glaucoma. It is important to be aware of the differential diagnosis so that other conditions that can present with visual field defects and/or optic neuropathy can be detected and dealt with promptly. External ocular, retinal, optic nerve, systemic and visual pathway problems can present with features of glaucoma. A careful history and thorough examination will guide the clinician to making the correct diagnosis. Particular emphasis should be made on the pattern of visual field defects, the optic disc cupping versus palor and whether the disc appearance correlates with the visual field defect. Timely medical advice should be sought to avoid sight/life-threatening conditions in some of these cases.

• 2224

**Counselling in advanced glaucoma**

ANSARIE

*Maidstone*

The loss of useful vision can be a very emotional experience for the patient and a massive challenge for the clinician. More time and a team-orientated approach is vital in order to support the patient through such a crisis. Disciplines such as counselling are an essential part of the management process for a patient with impaired vision and they can give advice on matters such as daily living, benefits, support groups and safety around the house. We have learnt that a separate multi-disciplinary consultation away from the hustle and bustle of the main clinic is very helpful for the patient, relatives and carers.

**SIS: ME and retinal disease****• 2231****ME and Retinal Disease***AMBATTI J  
Lexington***ABSTRACT NOT PROVIDED****• 2232****ME and Visual Acuity***TADAYONI R  
Paris***ABSTRACT NOT PROVIDED****• 2233****ME and RVO***LOEWENSTEIN A  
Tel Aviv***ABSTRACT NOT PROVIDED****• 2234****ME and Uveitis***LIGHTMAN S  
London***ABSTRACT NOT PROVIDED**

## • 2241

**Rationale for a diagnostic approach**

GAVARD-PERRETA A (1), ROBERT PY (2)

(1) *Nice*

(2) *Limoges*

Purpose: to propose a diagnostic approach for clinicians who manage a patient with non Graves' orbital inflammation. Patients&Methods: a review of the diagnostic process for 61 patients with diagnosis of orbital inflammation between January 2002 and January 2013 at Nice and Limoges University Hospitals. Results: sixty one patients presenting with orbital inflammation were included. More common presentations were orbital fat inflammation (60%), myositis (26%) and lacrimal gland enlargement (18%). In few cases, optic nerve and sclera were involved (respectively 9.8 and 3.2%). The final diagnostic was idiopathic orbital inflammation (25 patients), specific orbital inflammation (17 patients) and orbital lymphoma (19 patients). Thirty six patients had biopsy first and 25 patients did not, in first, but only after failure to the initial therapy. Our results showed that doing biopsy in the initial management of orbital inflammation provides a more great number of specific diagnosis than doing treatment tests directly after blood tests or CT scans ( $p > 0.01$ ). Conclusions: The diagnostic process of orbital inflammation must include blood testing, imaging of the orbit and sinuses, and biopsy in first intention as far as possible.

## • 2242

**Clinics, biopsy and pathology**

ROBERT P

*CHU Dupuytren, Limoges*

Etiological diagnosis of orbital inflammation drives the general handling, states on specific/non specific status of inflammation, and may lead to simple survey of long-lasting immunosuppressive treatment. Handling a orbital patient is always challenging for the clinician, but specific signs from clinical exam, imaging, biological checkup or histological samples may be critical. Clinical exam of the orbit must be directed towards ocular inflammation, sensitive and oculomotor signs, optic nerve function, and comparative orbital morphology. Imaging can disclose vascular, inflammatory, compressive, or bone-destructive lesions. Biological and systemic checkup must target signs of granulomatous, vasculitis, infectious, hematological and immunological diseases. Fine-needle biopsy is still controversial since it allows a first histological exploration under local anesthesia, but may be at risk of bleeding, perforating a noble organ (globe, muscle, optic nerve, lacrimal gland) or misdiagnosing a heterogenous or small lesion, when compared to explorative biopsy under general anesthesia. Progresses on immunohistochemistry and histological markers have made the proportion of "non-specific" orbital inflammation decrease these 10 last years. We review these techniques through demonstrative clinical cases.

## • 2243

**Inflammatory orbital lesions (IOI)**

DE KEIZER RJW (1), BIJLSMA WR (2)

(1) *Ophthalmology UZA, Edegem*

(2) *Ophthalmology, UMC Utrecht, Utrecht*

Idiopathic orbital inflammation is a diagnosis by exclusion and therefore a rather heterogeneous patient group. By definition the terms exclude lesions with an identifiable aetiology as bacterial, fungal and parasitic infections. Many classification schemes for IOI exists, but we didn't know which one is best so we like to share with the audience the outcome of our quest for the best classification system for idiopathic orbital inflammation. The classification systems could be tested for the five properties of a good classification system being Reliability, Feasibility, Face validity, Content validity, Distinction. A combined histology/imaging localization classification system is best for idiopathic orbital inflammation. The presentation will be including the topics idiopathic inflammatory orbital disease, which has to differentiate from the vasculitis and isolated sclerosing pseudotumor. The new classification schedules of the international vasculitis group will be shortly outlined. Also some research findings, where several attempts were done, to find relations with

## • 2244

**Orbital biopsies - the pathologist's perspective**

COUPLAND S

*Liverpool*

The orbital biopsies received by pathologists vary in size considerably according to surgical technique, suspected clinical diagnosis and subsequent surgical management. Presented is an overview of recommended "dos" and "don'ts" for ophthalmologists and their trainees when sending orbital samples to the pathology laboratory. This will include even the most simple aspects, such as adequate completion of the request form, communication with pathologist prior to sample sending, discussion about differential diagnoses and which one to prioritise initially during specimen workup, transport media and cassette recommendations, sample processing in the lab, and the variety of morphological, immunohistochemical and genetic testing available. This will be a "simple hands on" approach to orbital biopsies, with an opportunity for interaction by the audience and clarification of any unanswered questions.

**Joint Meeting: Improved monitoring and treatment of endogenous uveitis is modifying disease management/outcomes I: VKH disease**
**• 2251**
**Pathophysiology of the development of intraocular inflammation in VKH**

*HERBORT C (1, 2)*

(1) *Centre for Ophthalmic Specialized care b(COS), Lausanne*

(2) *University of Lausanne, Lausanne*

Choroiditis has recently been classified and subdivided into choriocapillaritis when the impact of inflammation is at the level of the choriocapillaris and into stromal choroiditis when the inflammation is situated in the choroidal stroma. The choroidal stroma can be the chance localization of an infectious or non-infectious systemic disease such as tuberculosis or sarcoidosis and is then termed secondary stromal choroiditis. The situation is different in Vogt-Koyanagi-Harada disease (VKH)(and birdshot retinochoroiditis) where the target of the inflammatory reaction is selectively situated in the choroidal stroma and should therefore be called primary stromal choroiditis. The choroidal inflammation develops first silently while it is still confined to the stroma. Probably this phase corresponds to the proromal stage. It is only when the inflammation spills over, usually in an explosive fashion, into neighbouring compartments such as the retina, optic disc and vitreous that the disease becomes clinically apparent. The fact that the choroidal stroma is the primum movens means that the follow-up of choroidal inflammation by ICGA is crucial and that therapy has a good impact as long as sustained ICGA assisted therapy is applied

**• 2252**
**ICGA features in VKH**

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Indocyanine green angiography (ICGA) is a highly sensitive method to evaluate choroidal inflammatory lesions. We present standardized ICGA findings in Vogt-Koyanagi-Harada (VKH) disease. Six ICGA signs including choroidal perfusion inhomogeneity, early hyperfluorescent stromal vessels, hypofluorescent dark dots (HDDs), fuzzy or lost pattern of large stromal vessels, disc hyperfluorescence, and diffuse late choroidal hyperfluorescence identified earlier were analyzed. The most constant findings present in the acute phase were four: early hyperfluorescent stromal vessels, HDDs, fuzzy/ lost pattern of large stromal vessels and disc hyperfluorescence. These angiographic signs represent a precise and universal tool to precisely assess choroiditis and follow-up VKH patients in the acute phase as well as in subacute disease, knowing that treatment of occult choroiditis in VKH is the key to avoid sunset glow fundus and even to "heal" VKH cases when treatment is given early and vigorously during a prolonged period.

**• 2253**
**OCT for VKH**

*NAKAI K*

*Suita*

Background: The aim of this work was to investigate the choroidal morphologic changes of Vogt-Koyanagi-Harada (VKH) disease *in vivo* using high-penetration optical coherence tomography (HP-OCT) with a long-wavelength light source (1,060 nm). Methods: Six patients with VKH disease were included in this study: 12 eyes of six patients with treatment-naïve acute VKH in the first 6–12 months after diagnosis. HP-OCT was used to observe the deep choroid and sclera. The choroidal thickness was measured for 6–12 months in eyes with acute disease. Results: In 12 eyes with acute VKH disease, the baseline choroidal thickness was significantly ( $p < 0.0001$ ) greater than in controls. After treatment, the choroidal thickness decreased over time. However, the choroidal thickness increased markedly again in four eyes with recurrent disease. The mean thickness at 12 months was significantly less than the normal value ( $p < 0.0001$ ). Conclusions: Significant choroidal thickness changes underlie VKH disease, which progress over time. Objective measurement of the choroidal thickness using HP-OCT may be useful for longitudinal evaluation of VKH activity.

**• 2254**
**Prognostic factors for clinical outcomes in patients with Vogt-Koyanagi-Harada disease treated with high-dose corticosteroids**

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Purpose: To determine prognostic factors in patients with Vogt-Koyanagi-Harada (VKH) disease who were treated with high-dose corticosteroids. Methods: Analysis of 87 patients (174 eyes). Results: Chronic recurrent presentation was significantly associated with more severe anterior segment inflammation at presentation as indicated by presence of mutton-fat keratic precipitates, anterior chamber reaction of 2+ or more, iris nodules and posterior synechiae, less exudative retinal detachment at presentation, more complications during the follow-up period and a worse visual outcome. The use of immunomodulatory therapy as first line therapy significantly reduced the development of complications in the whole study group and in initial-onset acute group and improved visual outcome in the whole study group. In the whole study group, final visual acuity of 20/20 was significantly associated with good initial visual acuity of >20/200 and age older than 16 years was significantly associated with the development of complications. Conclusions: Chronic recurrent VKH disease is significantly associated with more severe inflammation at presentation and more complications. Use of immunomodulatory therapy improves clinical outcome.

• 2255

**Vogt-Koyanagi-Harada disease: Sunset-glow fundus is not a fatality any more**

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Vogt-Koyanagi-Harada disease (VKH) is a primary stromal choroiditis, meaning that the inflammation is exclusively originating in the uvea and particularly in the choroid. Therefore management of the disease should include indocyanine green angiography (ICGA), the only way to monitor choroidal subclinical disease after the acute disease has regressed and is no more clinically apparent. Because this subclinical disease is progressing in an occult smoldering fashion, it is not astonishing that in close to 100% of studies evolution towards sunset glow fundus (SGF) is the rule. Thanks to ICGA it is now possible to detect occultly evolving choroiditis in VKH and adapt therapy accordingly. When ICGA assisted therapy of VKH is used, evolution towards SGF can be prevented showing that this is not the normal evolution in VKH but the result of insufficiently treated VKH. The purpose of this work was to show that close monitoring of choroiditis in VKH using ICGA can modify the phenotype of VKH by preventing the development of sunset glow fundus.

• 2256

**The outcomes of mycophenolate mofetil therapy combined with systemic corticosteroids in acute uveitis associated with Vogt-Koyanagi-Harada disease**

ABU EL ASRAR A

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Purpose: To study the effectiveness of mycophenolate mofetil (MMF) as first-line therapy in acute uveitis associated with Vogt-Koyanagi-Harada (VKH) disease. The outcomes in this group were compared with those of another group of patients who were treated with corticosteroid monotherapy. Methods: 19 patients(38 eyes) diagnosed with acute uveitis associated with VKH were included prospectively. Results: The mean follow-up period was  $27.0 \pm 11.1$  months. Corticosteroid-sparing effect was achieved in all patients. Ten patients discontinued treatment without relapse of inflammation. Visual acuity of 20/20 was achieved by 37.5% of the eyes in the corticosteroid group and by 73.7% in the corticosteroid + MMF group. Recurrent inflammation of 3 times or more was reduced significantly in the corticosteroid + MMF group as compared to corticosteroid group. Development of all complications was significantly higher in the corticosteroid group (42.6%) compared to the corticosteroid + MMF group (7.9%). None of the eyes in the corticosteroid + MMF group developed "sunset glow fundus". Conclusions: Addition of MMF as first-line therapy to corticosteroids improves clinical outcomes in patients with acute uveitis associated with VKH disease.

• 2261

**Oxidative-induced RGC-5 cell death culture involves mitochondrial dysfunction and can be attenuated by hydrogen sulphide**

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Exposure of cells in culture to light (1000lux, 400-760nm) results in cell death but only if their mitochondria are functional. A comparison between the death mechanisms of RGC-5 cells in culture caused by light or hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) showed them to be almost identical involving the stimulation of ROS and the activation and cleavage of both poly(ADP-ribose) polymerase-1 (PARP-1) and apoptosis-inducing factor (AIF) but not caspase-3. Their death processes are inhibited by similar substances that included hydrogen sulphide (H<sub>2</sub>S). The protective mechanism for H<sub>2</sub>S appears to involve the preservation of mitochondrial function deduced from the analysis of ATP, the mitochondrial membrane potential, the release of cytochrome c and the analysis of oxidative phosphorylation components in cells exposed to H<sub>2</sub>O<sub>2</sub>. This supports the notion that one way for H<sub>2</sub>S to act as a protective agent is to channel electrons into the electron transport chain of mitochondria.

• 2262

**Advancing age and mitochondrial dysfunction impair optic nerve recovery after IOP challenge**

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Glaucoma prevalence and incidence rise non-linearly with advancing age. The reasons for this may be multifactorial but this talk will demonstrate how advancing age and mitochondrial dysfunction in mice render the inner retina increasingly vulnerable to injury induced by short-term IOP elevation. We will then provide data from a cohort of open angle glaucoma patients demonstrating an impairment in complex-I-driven respiration and ATP synthesis, compared to age-matched controls. Put together these observations suggest that ageing and mitochondrial impairment render retinal ganglion cells vulnerable to IOP injury and this may predispose some individuals to open angle glaucoma.

• 2263

**The expanding clinical spectrum of dominant optic atrophy**

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(2) Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne

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Autosomal dominant optic atrophy (DOA) is the most common inherited optic nerve disorder in the population and OPA1 is the major causative gene, accounting for about 60% of families. Visual loss starts in early childhood secondary to the highly tissue-specific loss of one cell type – the retinal ganglion cell. Although progressive visual failure remains the defining feature of DOA, we recently described the expanding phenotypic spectrum associated with OPA1 disease. Up to 20% of mutational carriers developed a more severe "DOA+" phenotype characterised by prominent neuromuscular features such as deafness, myopathy, peripheral neuropathy, ataxia, and chronic progressive external ophthalmoplegia. Interestingly, we found a three-fold increased risk of developing the more severe DOA+ phenotype with missense OPA1 mutations involving the GTPase domain compared with other mutational subgroups. Histochemical and molecular characterisation of skeletal muscle biopsies revealed the presence of cytochrome c oxidase deficient fibres and clear evidence of mitochondrial DNA instability. Dissecting the disease mechanisms leading to optic atrophy and multisystem tissue involvement in DOA will have important therapeutic implications.

• 2264

**Sensorineural hearing loss in OPA1-linked disorders**

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About 20% of OPA1 mutation carriers have complicated forms of dominant optic atrophy associating extra-ocular features. Reviewing the files of 327 patients with an OPA1 mutation, we found 21 deaf patients (6.4%). In 10 patients deafness was detected under age 20, in 3 patients over age 20 and in 8 patients the age of onset was unknown. The severity of deafness ranged from mild and moderate. Audiological tests supported the diagnosis of auditory neuropathy in 8 cases. Seven different OPA1 mutations were identified in deaf patients. Three mutations, p.Arg445His, p.Gly401Asp and p.Leu243\*, have been previously reported in patients with deafness. Two mutations, p.Val291\_Phe328del and p.Ile463\_Phe464dup, have been reported in patients with DOA without deafness. Finally, two novel OPA1 mutations, p.Arg437Glu and p.Ala357Leufs\*4 were found. In the majority of patients with DOA and deafness, visual impairment occurred during the first decade while deafness appeared later. However, in 54% of patients deafness started prior to visual abnormalities. These observations suggest that audition should be carefully tested, including a specific search for auditory neuropathy in OPA1 mutation carriers.

**• 2265****The unsolved genetics of LHON: Beyond mtDNA primary mutations what else?***CARELLI V(1, 2)*(1) Dipartimento di Scienze Biomediche e NeuroMotorie, Università di Bologna,  
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(2) IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna

Leber's hereditary optic neuropathy (LHON) is a blinding disorder recognized as the most frequent mitochondrial disease. LHON is associated with mtDNA point mutations in complex I subunit genes, but remains characterized by poorly understood features such as tissue specificity, incomplete penetrance and male prevalence. Environmental factors (tobacco and alcohol) and specific mtDNA backgrounds (haplogroup J) are confirmed to play a role in modulating penetrance. We have also demonstrated that mitochondrial biogenesis and mtDNA copy number are major determinants on penetrance and gender difference in LHON. We are currently pursuing a multilayered "tour de force" to identify genetic modifiers by combining traditional tools, such as linkage analysis, with high-throughput techniques, such as MitoExome sequencing, functional and tag SNPs genotyping and microarray expression studies. Our results indicate that penetrance in LHON is modulated by polymorphisms in different genes rather than by a single mutation. Co-variates (age, sex, smoke and mtDNA copy number) have been instrumental to generating a list of candidate genes for which validation is currently ongoing.

## SIS: Clearance systems in the RPE cells-implications to AMD

### • 2271

#### The endolysosomal system and AMD: Insights from a novel genetically engineered mouse model

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In phagocytic cells, the endolysosomal system is a crucial regulator of both phagocytosis and autophagy, thereby helping to maintain cellular homeostasis. Prime examples of this are retinal pigmented epithelial (RPE) cells, which are not only among the most actively phagocytic cells, continuously phagocytosing shed photoreceptor outer segments (OS), but also are post-mitotic cells having high metabolic activity and a high rate of autophagy. For proper RPE function, and to ensure the functional integrity of the neural retina, both phagocytosis and autophagy need to be in balance. We show that in the RPE, bA3/A1-crystallin is required for normal lysosomal-mediated waste removal. Our studies suggest that bA3/A1-crystallin acts via the AMPK-mTORC1 signaling pathway. Based on our findings, we postulate that loss of bA3/A1-crystallin inhibits these signaling pathways, leading to a defect in the V-ATPase-mediated acidification of the lysosomal machinery. Since bA3/A1-crystallin has been reported to be present in human drusen and a possible role for lysosomes in age-related macular degeneration (AMD) has been suggested, it is possible that perturbation of normal phagocytosis/autophagy in RPE could cause some manifestations of AMD.

### • 2272

#### Crosstalk of proteasomes and autophagy in RPE cells

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The pathogenesis of AMD involves impaired protein degradation in RPE cells. The ubiquitin-proteasome pathway and the lysosomal pathway including autophagy are the major proteolytic systems in eukaryotic cells. Prior to proteolysis, heat shock proteins (HSPs) attempt to refold stress-induced misfolded proteins and thus prevent the accumulation of cytoplasmic protein aggregates. Recently, p62/sequestosome 1 has been shown to be a key player linking the proteasomal and lysosomal clearance systems. The aging characteristics of the RPE involve lysosomal accumulation of lipofuscin and extracellular protein aggregates drusens. Molecular mechanisms behind protein aggregations are weakly understood. There is intriguing evidence suggesting that p62, together with autophagy, seems to have a role in the pathology of different degenerative diseases. In ARPE-19 cells proteasomal inhibition caused accumulation of p62 bound irreversibly to perinuclear protein aggregates. The addition of the AMPK activator AICAR was pro-survival and promoted cleansing by autophagy of the former complex. Human AMD donor samples showed strong p62 accumulation in the drusen rich macula revealing impaired autophagy flux.

### • 2273

#### A novel role for the immunoproteasome in retinal function

FERRINGTON D

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The immunoproteasome (i-proteasome) is a protease that is abundant in cells of the immune system and is known to generate peptides for MHC class I presentation. I-proteasome is also present in tissues outside the immune system, including retina and RPE cells, suggesting alternative functions are possible. We have previously shown that i-proteasome is upregulated in the retina of donors with AMD, in response to acute injury in the retina and brain, and chronic oxidative stress in cultured RPE. These results suggest a novel role in the cellular stress response. Our recent focus has been on defining how i-proteasome is involved in regulating two key stress response pathways, NFkB and autophagy. To investigate i-proteasome's role, we used RPE cells derived from WT and KO mice lacking either one (Lmp2<sup>-/-</sup>) or two (Lmp7<sup>-/-</sup>/mec1<sup>-/-</sup>) i-proteasome catalytic subunits. Results show that cells deficient in the LMP2 subunit demonstrate differences in the alternative pathway of NFkB signaling, as well as a diminished response to autophagic stimuli. Thus, i-proteasome upregulation with AMD, and with chronic and acute injury, may be a compensatory response that is required to help regulate these two stress response pathways.

**• 2321****Why what you have been taught about the optic disc may not be entirely true***CHALIHAN B**Dalhousie University, Halifax*

Clinicians evaluate neuroretinal rim health according to the appearance of the optic disc, the clinically visible surface of the optic nerve head (ONH). Recent anatomic findings with optical coherence tomography challenge the basis and accuracy of current rim evaluation for 3 reasons: (1) The DM is rarely a single anatomical structure or an identifiable junction, such as the inner edge of border tissue. In most eyes it corresponds variably to multiple anatomical structures.(2) In some regions of all ONHs, Bruch's membrane extends internally beyond the DM (towards the centre of the ONH) and is both clinically and photographically invisible. Since in these areas the outer border of the rim is the termination of Bruch's membrane and not the more external DM, the rim is narrower than that with clinical or photographic evaluation. (3) Because current rim width measurements are made in a fixed plane without reference to the orientation of the rim tissue, for the same number of axons, the rim width will be greater in cases where the orientation of the rim tissue is more horizontal (for example in the temporal sector of tilted optic discs) compared to when it is more perpendicular. This presentation will review and interpret ONH anatomy detected with optical coherence tomography pertaining to optic disc examination and demonstrate why a paradigm change for clinical assessment of the optic nerve head is now necessary.

• 2411

**Postoperative quality of life in macula-off rhegmatogenous retinal detachment patients and its relation to visual function**

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**Purpose** To assess which aspects of postoperative visual function (visual acuity, contrast acuity, or color confusion) are correlated with visual function-related quality of life measurements after successful surgery for macula-off rhegmatogenous retinal detachment (RRD).

**Methods** In patients with a primary macula-off RRD, best correct visual acuity (BCVA), color confusion indices (CCI saturé et désaturé), and contrast acuity were measured at 12 months postoperatively in both the RRD eye and the fellow control eye, and the 25-item National Eye Institute Visual Function Questionnaire (VFQ-25) was filled out.

**Results** Forty-five patients with a primary unilateral macula-off RRD were included. There was a significant difference between operated and fellow control eyes with regard to LogMAR BCVA ( $P < 0.0001$ ), Log contrast acuity ( $P < 0.0001$ ), saturé CCI ( $P = 0.018$ ), and désaturé CCI ( $P = 0.018$ ). Correlations were observed between the composite VFQ-25 score and postoperative LogMAR BCVA ( $R = -0.53$ ,  $P < 0.0005$ ) and Log contrast acuity ( $R = 0.47$ ,  $P = 0.001$ ). In addition, correlations were found between postoperative LogMAR BCVA and Log contrast acuity and the following subscales: driving, near acuity, role difficulties, and general vision. Also, a correlation between the subscale mental health and LogMAR BCVA was found. No correlations were found between any VFQ-25 scales and the CCIs.

**Conclusion** Postoperative BCVA, contrast acuity and color confusion in operated macula-off RRD eyes were significantly worse compared to their fellow control eyes. Several vision-related quality of life parameters were highly correlated with postoperative BCVA and contrast acuity.

• 2412

**Posterior vitreous detachment in highly myopic eyes undergoing vitrectomy**

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**Purpose** To review the PVD status in highly myopic eyes requiring vitreoretinal surgery, using intraoperative observation

**Methods** In a retrospective study, patients presenting with high myopia with refractive error <-6.00D, axial length >26mm or fundus signs of high myopia, who underwent their first intracocular vitreoretinal surgery in our department between 2009 and 2012 were included. Standard 25G-pars plana vitrectomy was performed for all, with or without staining. The main outcome was the intraoperative identification of a PVD. Age at the time of surgery and preoperative slit-lamp observation of PVD were also collected.

**Results** Ninety-six eyes of 95 patients were included. Eyes were categorized by disease: myopic foveoschisis (FVS), epiretinal membrane (ERM), macular hole (MH), macular hole retinal detachment (MHRD) and rhegmatogenous retinal detachment (RD). Mean age was 62.15 years (range 29-95). RE was available for 67 eyes: mean value -13.9D. Mean AL on 38 eyes was 29.2mm. A PVD was found in 52.1% of the 96 eyes, and a dye was used in 81 eyes. In MH, FVS, MHRD, ERM and RD groups, the PVD rate were respectively 10%, 14.3%, 42.9%, 74.2% and 85%. Reliability of preoperative slit lamp examination in detecting the PVD showed a 70% sensitivity, 76% specificity, 75% positive predictive value and 70% negative predictive value.

**Conclusion** In highly myopic eyes when ERM and RD groups the PVD rate was not so far from emmetropic eyes, in case of RDMH, MH and FVS this rate was found very low. Nearly 90% of highly myopic eyes with FVS or MH have their posterior vitreous cortex still attached. As the assessment of PVD in these eyes is very challenging, the use of any dye or visualization agent during these surgeries seems then advisable.

• 2413

**Epiretinal membrane surgery in highly myopic eyes a case control study**

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**Purpose** To evaluate the anatomic and functional outcomes of epiretinal membrane peeling surgery in highly myopic eyes and to compare them with non-highly myopic controls. Design: case control study in a retrospective cohort of 1776 patients undergoing surgery for epiretinal membrane alone or combined with cataract extraction.

**Methods** Fifty-seven highly myopic eyes from 57 patients with axial length over 26 mm were included in the study. For each case studied, we selected from the same cohort one control matched for preoperative acuity, central macular thickness, and surgical technique. Best-corrected visual acuity (BCVA), decrease of central macular thickness (CMT), impact of axial length on postoperative visual recovery and postoperative complications were studied.

**Results** The postoperative best-corrected visual acuities improved by 0.37 LogMar in highly myopic eyes versus 0.39 LogMar in controls (NS). The mean postoperative foveal thickness decrease was 110 µm in highly myopic versus 77 µm in controls ( $p=0.0585$ ). We did not find any correlation between axial length and improvement of BCVA. We had no difference in the incidence of complication between the two groups.

**Conclusion** ERM peeling gave good anatomical and functional results in highly myopic patients. The axial length has no impact on visual recovery, foveal thickness decrease and postoperative complications.

• 2414

**Baseline characteristics and vitreoretinal interface features in patients with vitreomacular traction associated with macular hole from the MIVI-TRUST clinical program**

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**Purpose** There is limited published evidence on demographic and vitreoretinal interface (VRI) characteristics of patients with vitreomacular traction in association with macular hole (VMT+MH). Establishing insights into the characteristics of untreated VMT+MH may contribute to a better understanding of the burden of VMT diseaseTo describe baseline patient characteristics and VRI features in patients with VMT+MH included in the phase 3 ocriplasmin studies

**Methods** Two randomized, double-masked, placebo-controlled trials designed to determine efficacy and safety of ocriplasmin for the treatment of VMT comprising 652 patients. Key baseline characteristics included patient demographics (age, gender); eye-disorder (time since VMT diagnosis, visual acuity (VA) study eye (SE); non-study eye (NSE), pseudophakia and/or ERM); VRI features (MH width; vitreous separation), VFQ-25 composite score

**Results** VMT+MH was present in 153 patients (23.5%). Baseline characteristics were: 68.7 years (SD 7.1); 75.8% female. Time since diagnosis: 62 days (SD 131); VA: SE 55.9 (SD 10.5); NSE 77.8 (SD 14.3). Pseudophakes: 20.9%, concomitant ERM: 15.8%. Min-max hole width: 272.7 (SD 127.7), vitreous adhesions attached at fovea and optic nerve (89.5%); VFQ-25 composite score: 80.3 (SD 14.6)

**Conclusion** This analysis reports baseline characteristics of patients with VMT+MH, which can be used to demonstrate differences in VMT patients without MH

• 2415

**Baseline characteristics predictive of pharmacologic vitreomacular adhesion resolution in the ocriplasmin MIVI-TRUST program**

GANDORFER A

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**Purpose** The ocriplasmin MIVI-TRUST program included two phase III, multicenter, randomized, double-masked trials to determine the efficacy and safety of ocriplasmin for the treatment of vitreomacular traction (VMT). We sought to identify baseline characteristics that were predictive of pharmacologic vitreomacular adhesion (VMA) resolution at Day 28 after an injection of ocriplasmin or placebo.

**Methods** Patients with OCT-confirmed VMA were randomized to receive a single intravitreal injection of ocriplasmin 125 µg (n=464) or placebo (n=188). The primary end point was VMA resolution at 28 days post-injection. A multivariate regression analysis was performed to determine independent baseline anatomic and non-anatomic characteristics that were significantly associated with pharmacologic VMA at Day 28.

**Results** Pharmacologic VMA resolution at Day 28 was observed in a significantly larger proportion of patients in the ocriplasmin group (26.5%) compared with placebo (10.1%; p<0.001). Independent baseline anatomic characteristics predictive of VMA resolution included the presence of a full-thickness macular hole (equivalent to stage II; p=0.019), VMA diameter ≤1500 µm (p<0.001), phakic lens status (p<0.001), and the absence of an epiretinal membrane (p<0.001). Independent non-anatomic baseline characteristics predictive of VMA resolution included treatment with ocriplasmin (p<0.001) and age <65 years (p<0.001).

**Conclusion** The identification of specific baseline anatomic characteristics predictive of response is informative in the consideration of treatment options for patients with VMT.

*Commercial interest*

• 2416 / F027

**Baseline characteristics and vitreoretinal interface features in patients with vitreomacular traction without macular hole from the MIVI-TRUST clinical program**

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(4) *Xintera, Leuven*

**Purpose** There is limited published evidence on demographic and vitreoretinal interface (VRI) characteristics of patients with persistent vitreomacular traction (VMT). Establishing characteristics of untreated VMT may contribute to a better understanding of the burden of VMT diseaseTo describe baseline characteristics and VRI features in patients with persistent VMT included in the phase 3 ocriplasmin studies. This analysis reports on VMT patients without macular hole (MH)

**Methods** Two randomized, double-masked, placebo-controlled trials designed to determine efficacy and safety of ocriplasmin for the treatment of VMT comprising 652 patients. Key baseline characteristics included patient demographics (age, gender); eye-disorder (time since diagnosis, visual acuity (VA) study eye (SE), non-study eye (NSE), presence of pseudophakia or ERM); VRI features (type of focal VMA, vitreous separation); VFQ-25 composite score

**Results** VMT without MH was present in 499 patients (76.5%). Baseline characteristics were: 72.6 years (SD 9.8); 62.7% female. Time since diagnosis was: 268 days (SD 547); VA: SE 66.8 (SD 11.2); NSE 73.5 (SD 16.5). Pseudophakes: 38.7%, concomitant ERM: 46.3 %. The majority of patients presented with focal adhesion ≤1500 microns (69%) and with vitreous adhesions attached at fovea and optic nerve (92%); VFQ-25 composite score: 78.0 (SD 15.2)

**Conclusion** Baseline characteristics and vitreoretinal Interface features in patients with vitreomacular traction without macular hole from the MIVI-TRUST Clinical Program

• 2417 / F026

**Intravitreal drug dispersion and needle gauge**

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**Purpose** Intravitreal injections (IV) have become the main treatment for many ocular diseases.. At present, IV are supplied with a 27G, 30G needles. Reduced ocular discomfort and scleral penetration forces have been reported after using smaller gauge needles. However, smaller inner diameter implies increased fluid speed and intraocular turbulence that may result in retinal harm

**Methods** IV were injected with 0.05 mL Indian ink using 27, 30 and 32 G needles. After the injection the eyes were divided in groups and the vitreous was photographed. Three masked observers scored Indian ink dispersion within the vitreous gel from 1 (completely localized) to 4 (complete dispersion).

**Results** Intra-observer and inter-observer reproducibility was between 0.78 and 0.95 and between 0.93 and 0.95, respectively (intra-class correlation coefficient). The average Indian ink dispersion scores for 27, 30 and 32 G needles were 3.4, 2.5 and 1.8 respectively (p=0.07 for 27 vs. 32 G; p= 0.71 for 27G vs. 30G; p=0.15 for 30G vs. 32G; Student t test for unpaired data)

**Conclusion** The inner flow is four times faster in thinner needles, considering that the plunger slides with similar speed. We might expect that flow through the thinner needle would induce more turbulence within the vitreous gel; however, the pattern of intravitreal distribution of Indian ink suggests that the injected fluid and the turbulence were confined to a small bag in the area where smaller gauges were used. The results of this work suggest that 32G needles do not increase turbulence of injected fluid. Limited turbulence may be of even greater interest in elderly eyes with liquefied vitreous gel, reducing mechanical retinal damage

## SIS: Myths and misconceptions in glaucoma

### • 2421

#### Myths and misconceptions about glaucoma blindness

HONDEGHEM K

*Glaucoma department, ZNA Middelheim, Antwerp*

Blindness is a major global problem, with many associated socioeconomic impacts. Glaucoma is responsible for much of this worldwide blindness. Glaucoma is the leading cause of irreversible vision loss. We will take a closer look at ten common myths and misconceptions about (glaucoma) blindness. We will also briefly discuss some exciting new technologies that can be a tremendous aid to the blind.

### • 2422

#### Misconception about the intraocular pressure

POURJAVAN S

*Ophthalmology, Brussels*

There are still some strong misconceptions about the intraocular pressure among the specialists. Some of them are the conclusion of the large clinical randomized studies. The myth of 21 mmHg is one of the most common misconceptions. This talk will cover some of the large randomized studies and review critically the conclusions.

### • 2423

#### Generics are the same or similar?

STEVENS AM

*University Clinic, Gent*

This presentation will briefly summarize the requirements for bio-equivalence according to the European legislation (E.M.E.A.) The different requirements for systemic and non-systemic generics will then be clarified as well as their implications for topical ophthalmic medications. Six different elements that might interfere with therapeutic equivalence of brand and generic ophthalmic formulations are considered: inactive ingredients, bottle design and drop size, stabilizers, pH stability, temperature stability, and presence of particulate matter. A number of studies comparing brand and generic ophthalmic drugs will be presented. Finally, the cost saving aspect of generic ophthalmic drugs is analyzed with particular reference to the Belgian situation.

### • 2424

#### Myths and misconceptions in treatment adherence

KOTECHA A

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Once diagnosed, the glaucoma patient requires lifelong treatment, usually with topical IOP lowering medication. The aim of glaucoma treatment is to reduce IOP, a 'clinician-based' measure, and thus treatment has no obvious effect on the patient's vision or their ability to carry out every-day tasks. There is evidence to suggest that medication underuse occurs in patients with glaucoma. Deliberate medication underuse occurs when the patient is concerned with aspects relating to either the treatment safety, or treatment efficacy and necessity. In glaucoma, medication misuse may lead to inappropriate conclusions regarding treatment inefficacy, resulting in unnecessary changes and additions to treatment, and, more seriously, avoidable, irreversible visual loss. Improving patient adherence to their treatment is a complex issue influenced by a number of factors. This talk will explore some of the issues regarding glaucoma medication misuse and the role of the 'doctor-patient' relationship in promoting adherence to treatment.

## • 2431

**Novel strategies for angioregression at the ocular surface**

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Cologne

The normal human cornea is avascular. This avascularity is actively maintained by expression of antiangiogenic and antilymphangiogenic factors. Corneal neovascularization (NV) can occur after numerous corneal diseases and leads to decreased visual acuity and poor prognosis in subsequent corneal transplantation since corneal immune privilege is lost. We recently demonstrated that bevacizumab eye drops significantly inhibit progressive corneal angiogenesis. In contrast, limited treatment options exist for mature corneal vessels, which — because of their pericyte-covered vessel wall—do not depend on VEGF anymore. Here, we report on the short- and long-term visual acuity changes and changes in vascularized corneal area after e.g. feeder vessel coagulation combined with topical bevacizumab application for the treatment of mature pathological corneal NV. These angioregressive therapies may not only improve corneal transparency but also “preconditions” such a cornea for future keratoplasty.

## • 2432

**Mesenchymal stem cells and corneal regeneration**

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Mesenchymal stem cells (MSC) are the subject of intensive research for regenerative medicine purposes. MSC can be isolated from a broad range of tissues, allowing use in both autologous or allogeneic stem cell transplantation. Furthermore, these cells are readily bankable and their numbers can be expanded *in vitro*. MSC possess an impressive functional-repertoire as they are differentiated into a steadily growing number of cell types, including those found in the cornea. However the regenerative abilities of MSC extend beyond differentiation. MSC are able to modulate and suppress localised immune responses encouraging graft acceptance and reducing occurrences of GVHD/rejection. Furthermore they are able to encourage regeneration by the host tissue using a multitude of paracrine signalling pathways. MSC have previously been shown to promote corneal wound healing both with and without incorporation into the host tissue. MSC have been found to reside in the limbal and peripheral corneal stroma. Although their role in normal corneal homeostasis and wound healing is unknown there have recently been tantalising glimpses into their potential for substantial corneal regeneration.

## • 2433

**Results of a phase I/II clinical trial: standardized, non-xenogenic, cultivated limbal stem cell transplantation**

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Antwerp

We describe the results of a phase I/II clinical trial for standardized, non-xenogenic, cultivation and “no touch” surgical transplantation of limbal stem cell grafts. 18 eyes of 18 patients were transplanted with either autologous (n=15) or allogenic (n=3) limbo-amnion composite grafts that were generated using a standardized culture protocol free of xenogenic culture products and transplanted using a standardized “no touch” surgical technique. *In vitro* cellular outgrowth and phenotype of the cultivated graft was assessed prior to transplantation. Corneal neovascularization, central corneal opacity, pain, photophobia and visual acuity were investigated pre and post transplantation. The limbal epithelial cells showed an average outgrowth of 14.2mm by day 14. The majority of the cells displayed a progenitor phenotype: p63, CK14, desmoglein, ABCG2 bright and CK3/12 dim protein expression. The transplant recipients were followed up for a mean of 22 months. 12 out of the 18 transplant recipients were graded successful, giving an overall success rate of 67%. The ocular surface photographs for pre- and post stem cell transplantation, showed a significant ( $p=0.007$ ) reduction in the percentage area of corneal neovascularization.

## • 2434

**Transcriptional profiling of human corneal epithelium using laser capture microdissection and massive parallel sequencing**

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This study was conducted to perform global transcriptomic profiling and biomarker discovery on undisturbed human corneal epithelial subpopulations using a novel approach combining laser capture microdissection (LCM) and RNA sequencing. LCM facilitated the harvest of four cellular compartments along the corneal epithelial differentiation pathway: Stroma, basal limbal crypt (BLC), superficial limbal crypt (SLC), and cornea. Compartments were analyzed using massive parallel sequencing and bioinformatics. Specificity of LCM was confirmed by imaging, expression of epithelial markers, and gene ontology analysis of biological processes. Interestingly, many significant upregulated genes in SLC mapped to processes involved in regulation of vasculature like sFLT1. In contrast, BLC had many upregulated genes mapping to neurogenic and developmental processes. The primitive nature of BLC was confirmed by KEGG pathway analysis. We present full gene lists of unique expressed genes in both BLC and cornea representing candidate biomarkers. Possible novel regulators of limbal epithelial stem cells (LESCs) include Lrig1 and SOX9. The presented molecular insight in LESC biology is expected to be beneficial for both research and clinical translation.

**• 2435****Suitability of a fish scale-derived collagen matrix (FSCM) as artificial cornea**

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Purpose: A naturally occurring, easily obtainable fish scale-derived collagen matrix (FSCM) may be a cheap alternative to current keratoprostheses. We assessed its suitability by measuring light transmission, scattering, and immunogenicity. Methods: Light transmission and scatter characteristics of the FSCM were measured. The FSCM was implanted subconjunctivally in rats, to assess swelling and neovascularization, and subcutaneously to observe the cellular immune response. Results: The FSCM had a light scattering of  $\log(s) = 1.62$  and a light transmission of 90%. Local swelling and neovascularization appeared similar to an implant already used in glaucoma surgery. Both subcutaneous implants elicited an initial and mild immune reaction with only a few lymphocytes after 2.5 months, and did not induce immune sensitization. The FSCMs were well tolerated, while a small fibrous capsule was also observed. Conclusions: We demonstrate that the first prototype of this easily obtainable, naturally occurring FSCM has proper optical clarity, and low immunogenicity. These properties demonstrate its potential as a candidate for reconstructing the avascular cornea.

*Commercial interest*

## Course 9: The management of pigmented fundus lesions

### • 2441

#### Congenital and other pigmented fundus lesions

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Purpose: To describe congenital and pigmented fundus tumors except nevi and melanomas. Methods: We analyse diagnostic approaches of these tumors, their characteristics, the differential diagnosis and the follow up. Results: The main tumors include congenital tumors such as congenital hypertrophy of the retinal pigment epithelium (RPE), hamartoma of the RPE, combined hamartoma of the RPE and sensory retina. The other pigmented tumors are melanocytoma, adenoma or adenocarcinoma of RPE and pigmented choroidal metastasis. The differential diagnosis must be made from RPE hyperplasia, peripheral exudative haemorrhagic chorioretinopathy and pseudo tumoral age related macular degeneration. Conclusions: These tumors must be known to be differentiated from melanomas and choroidal nevi. Moreover, although these lesions are mainly benign in their clinical behavior, some cases can have ocular complications, slow growth or malignant transformations.

### • 2442

#### Suspicious choroidal naevi

KIVELÄ T  
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The speaker will summarize characteristics of and guidelines for referral of non-suspicious and suspicious choroidal naevi, based on review of literature and personal experience. Recognition and characterisation of choroidal naevi is based on fundus examination with the slit lamp and indirect ophthalmoscope, supplemented with ultrasonography (thickness, acoustic profile), optical coherence tomography (subretinal fluid, thickness) and fundus autofluorescence (subretinal fluid, orange pigment). Large to medium-sized melanomas are readily differentiated from naevi using these methods. The challenge lies in telling small melanomas from naevi. The mnemonic 'To Find Small Ocular Melanomas' reminds us to look for tumour Thickness of more than 2 mm, subretinal Fluid, visual Symptoms, Orange pigment and location of the tumour Margin at the optic disc. Each of these risk indicators approximately doubles the likelihood of growth and malignancy, but none of them is specific. Surveillance for growth, a biopsy, or both, may thus be needed after the initial evaluation.

### • 2443

#### Malignant melanoma of the uvea: Diagnosis, characterization and prognosis

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Purpose: To summarize available data on uveal melanoma biology, diagnosis and clinical characterization. Methods: Herein, we review diagnostic criteria, clinical characterization and therapeutic implications of the emerging molecular biology in posterior uveal melanoma/high risk indeterminate choroidal pigmented lesion. Results: The diagnosis of uveal melanoma is based on clinical examination with indirect ophthalmoscope and ultrasonography. The use of mnemonic "to find small ocular melanoma" is useful in risk stratification of small indeterminate melanocytic choroidal lesions. The use of optical coherence tomography and autofluorescence also help in identifying sub-retinal fluid and orange pigment. In the cytogenetic era, tumor-sampling procedures are becoming the main prognostic tools for uveal melanoma patients. Conclusions: Ophthalmic oncology is moving in the same direction as all other oncologic subspecialties, starting to determine the patient's risk using sampling-based laboratory techniques. However, it is essential to start treating patients earlier (in the premalignant tumor stage) and to develop consistent, selective and effective adjuvant therapy.

### • 2444

#### Malignant melanoma of the uvea: Radiotherapy techniques

DESJARDINS L  
Paris

Purpose Malignant melanoma of the uvea is a relatively radioresistant tumor. For this reason only radiotherapy techniques with precise location of the radiation doses are used. Methods Proton beam therapy has been used since 1975 by Gragoudas in Boston and in Europe since 1983. Because of the Bragg peak, the use of an accelerated proton beam allows delivering homogenous high dose to the tumor while sparing the normal tissue around. Brachytherapy is also widely used. Iodine 125 is a low energy gamma emitting isotope; the radiation is totally stopped by heavy metals like gold. The seeds need to be changed every two months because of its half life. Ruthenium 106 is a beta emitting isotope. Its low penetration prevents its use for the bigger tumors. With brachytherapy the dose of 90 grays is usually delivered to the top of the tumor while the base receives more than 300 grays. Results Tumor control is excellent with proton beam (less than 5% recurrence at 5 years) and good with brachytherapy (less than 10% recurrence at 5 years). Globe preservation is possible in more than 90% of treated patients.

**Course 9: The management of pigmented fundus lesions****• 2445****Malignant melanoma of the uvea: Surgical techniques***DAMATO B**Ocular Oncology Service, Liverpool*

Uveal melanoma can be excised by local resection or enucleation. Local resection comprises trans-scleral 'exoresection' and trans-retinal 'endoresection'. Variations of exoresction include: iridectomy; irido-cyclectomy; cyclo-choroidectomy and chorioidectomy. Resection of melanomas involving ciliary body and/or choroid is usually followed by adjunctive brachytherapy, which is administered immediately after the excision procedure or several weeks later, once the eye has healed. Such adjunctive radiotherapy prevents local recurrence while avoiding the need for wide safety margins, thereby reducing ocular morbidity. Endoresection is performed through a retinotomy over the tumour or after raising a retinal flap. Enucleation is undertaken in the conventional manner, using the surgeon's preferred implant. Primary local resection is performed when radiotherapy is likely to cause excessive morbidity, because of large tumour size or proximity to the optic disc. Secondary resection is undertaken as a treatment for local tumour recurrence or the toxic tumour syndrome after radiotherapy. Surgical resection provides ample tumor material for diagnosis and prognostication, which may in future be useful therapeutically.

## SIS: Inflammation and glaucoma: good or bad?

### • 2451

#### **Uveitis and glaucoma: A practical approach**

BODAGHI B

Paris

Uveitis is a sight-threatening condition with major therapeutic breakthroughs during the last decade. Control of inflammation is more frequently achieved. Therefore, blindness is often associated with secondary complications. Severe glaucoma is one of the most difficult situations to manage. Both inflammation and steroids may induce ocular hypertension in patients with uveitis. Most of cases are associated with anterior uveitis. New entities associated with viral infections have been identified during the last decade. Specific antiviral therapy is often sufficient to control both inflammation and high IOP. In noninfectious conditions, control of inflammation with immunosuppressors and biologic agents have dramatically changed the incidence of glaucoma in these patients. Prompt diagnosis and efficient treatment of uveitis have dramatically changed the outcome and long-term prognosis of uveitis. Glaucoma surgery remains rarely necessary but sometimes the only possible strategy.

### • 2452

#### **Autoimmune mechanisms in primary open angle glaucoma**

GRUS F

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In glaucoma, the elevated intraocular pressure cannot explain the disease in all patients. However, the pathogenesis of the disease is widely unknown. Autoimmune effects could play a role in the disease process. Several studies using Western blotting, mass spectrometry and microarrays could demonstrate significant and persistent up – and downregulations of immunoreactivities against ocular antigens. Furthermore, in animal models it could be shown that in principle the immune reaction itself is able to lead to retinal ganglion cell loss. A deposit of antibodies could be shown in the retinae of glaucoma patients. These findings could lead to a better understanding of the pathogenesis, but also to new immunomodulatory treatment options and diagnosis.

### • 2453

#### **Role of glial activation in neuroprotection of the retina**

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The effects of retinal injury on the neuronal and non-neuronal cell population of the injured and the fellow-uninjured retina were investigated in adult mice. Unilateral left eye retinal injury was induced by intraorbital optic nerve transection (IONT) or by laser-induced ocular hypertension (OHT). Both retinas were prepared as wholemounts and immunostained with Brn3a, Iba-1 and GFAP to identify, count and map the distribution of retinal ganglion cells (RGCs), microglia and astrocytes, respectively. OHSt was used to trace RGCs and to identify phagocytic microglia. By 2 wks after IONT or OHT, RGCs in the left retinas represented 20% of the original population. Following IONT, microglial cells in the left retinas increased with time from center to periphery and this response diminished with BDNF treatment, while in the fellow retinas phagocytic microglial cells appeared at 3 days but their numbers were not modified with vehicle or BDNF. Following OHT there was a marked macro and microglial reactivity in both retinas. Thus, IONT and OHT induce changes in the macroglia and microglia of the injured and contralateral uninjured fellow retinas. The gliotic response in the fellow retinas could be immune related.

### • 2454

#### **How inflammatory reactions affect POAG topical and surgical treatment?**

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There is a large body of evidence from clinical and experimental studies that the long-term use of topical drugs may induce inflammatory ocular surface changes. The high prevalence of symptoms and signs of ocular surface disease (OSD) in patients with glaucoma or OHT has been demonstrated in several clinical studies. In parallel, inflammatory changes of the ocular surface directly impact the effectiveness of antiglaucoma medical and surgical treatments. Ocular surface side effects impact the quality of life and are significant barrier to adherence in patients treated for glaucoma or ocular hypertension. Moreover, as conjunctival wound healing is a major determinant for the success of glaucoma filtering surgery, ocular surface inflammation is a well-known risk factor for surgery failure by subconjunctival fibrosis. Therefore, a better knowledge of ocular surface inflammatory changes with appropriate evaluation and management should thus become a new paradigm in glaucoma care.

• 2471

**Restoration of keratocyte cell phenotype through epithelial-stromal cell interactions in a 3D hydrogel model**

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**Purpose** In vivo, epithelial cells are connected both anatomically and functionally with stromal keratocytes. In vitro co-culturing aims to recapture this cellular anatomy and functionality by bringing together two or more cell types within the same culture environment. The aim of this study was to investigate epithelial-stromal cell signalling for the control of corneal stromal cell plasticity in 3D culture models.

**Methods** Corneal stromal cells were activated to their injury phenotype (fibroblasts) before being encapsulated in 3D collagen hydrogels. 3 different epithelial-stromal co-culture methods were then examined; epithelial explant transwell; the use of conditioned media. Using non-destructive monitoring tools we revealed how epithelial co-culturing affects stromal cell differentiation in terms of construct contraction and elastic modulus measurements in a 3D collagen hydrogel environment for prolonged culture periods. Cell viability, phenotype, morphology and protein expression was investigated to corroborate our mechanical findings.

**Results** It was shown that activated stromal cells retain their plasticity in vitro. Activated corneal stromal cells that were fibroblastic in phenotype were successfully reverted to a non-activated keratocyte cell lineage in terms of behaviour and biological properties; and then back again via TGF- $\beta$ 1 media supplementation. It was then revealed that epithelial-stromal interactions can be blocked via the use of wortmannin inhibition.

**Conclusion** A greater understanding of stromal-epithelial interactions and what mediates them offers great pharmacological potential in the regulation of corneal wound healing, with the potential to treat corneal diseases and injury whereby such interactions are vital.

• 2472

**Culture optimisation of CD34-positive corneal stromal stem cells and transdifferentiation into corneal epithelial cells**

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**Purpose** The integrity of the corneal epithelium is essential for clarity of vision. Under normal circumstances, superficial epithelial cells are shed into the tear film and regenerated by epithelial stem/progenitor cells located at the limbus. Damage or depletion of the limbal cells leads to conditions such as limbal stem cell deficiency (LSCD). Current treatment involves transplantation of limbal epithelial cells expanded ex-vivo; however cell numbers are limited. In this study, we identify a source of multipotent stem cells located in the corneal stroma that express CD34 upon isolation. These cells demonstrate an ability to transdifferentiate into corneal epithelial cells.

**Methods** CD34 expression diminishes during traditional tissue-culture plastic propagation; in this study we optimised culture conditions for maintained and efficient expansion of CD34+ cells. We investigated the role of CD34 in epithelial transdifferentiation of stromal cells, using siRNA gene knockdown and analysis by immunocytochemistry, flow cytometry and qPCR.

**Results** Early results suggest a three-dimensional environment and semi-solid medium intended for hematopoietic culture demonstrated extended CD34 expression. When cells were transferred to epithelial differentiation medium they showed an epithelial morphology, significantly increased cytokeratin 3 and 19 expression, and considerable upregulation of genes related to corneal epithelial cells (ABCG2, DeltaN63, LEF1, HES1, FRZB1, KRT19, DTC and CDH1).

**Conclusion** This work will help produce methodologies to create cell banks for generation of corneal epithelium, from a corneal stromal stem cell source, leading to improved surgical and visual outcomes in LSCD patients.

• 2473

**Small molecule induction promotes corneal epithelial cell differentiation from human pluripotent stem cells**

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**Purpose** Corneal epithelium is maintained by limbal stem cells, and their transplantation has been used to treat limbal stem cell deficiency (LSCD). However, this is only possible if enough healthy limbal tissue is available. Thus, novel cell sources for treating LSCD are needed. Human induced pluripotent stem cells (hiPSCs) provide unique opportunities for differentiation of limbal and corneal epithelial cells for cell transplants.

**Methods** In order to improve the efficiency and reproducibility of hiPSCs differentiation towards corneal epithelial progenitor cells, signaling cues active during ocular surface ectoderm development was replicated with two small-molecule inhibitors in combination with growth factors. The extent of differentiation was evaluated by following the expression of key markers using immunocytochemistry and qPCR at several time-points.

**Results** Small-molecule induction down-regulated the expression of pluripotency marker OCT4 while up-regulating the eye-field transcription factor PAX6. Protein expression of the corneal epithelial progenitor marker p63 was greatly enhanced, with up to 95% of cells being p63-positive after five weeks of differentiation. Finally, after a total of six weeks in differentiation culture, the two markers specific to terminally differentiated corneal epithelium, cytokeratins 3 and 12, were expressed in an average of 35% and 71% of cells, respectively.

**Conclusion** In contrast to all earlier studies, corneal epithelial cells were differentiated in serum-free culture conditions without the use of amniotic membrane or other undefined culture substrates. This highly efficient differentiation method could potentially be used for treating LSCD in the future.

*Commercial interest*

• 2474

**Computational model of Ca2+ wave propagation in human retinal pigment epithelium**

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**Purpose** Ca2+ signaling is relevant to most biological functions. In retinal pigment epithelium (RPE) a significant Ca2+ wave is produced by mechanical stimulation. To understand this process in detail, we modeled Ca2+ wave propagation in ARPE-19 cells.

**Methods** Mechanically induced Ca2+ wave was recorded from ARPE-19 cells by Ca2+ imaging. Based on the measurements, a mathematical model was constructed. The model assessed Ca2+ wave propagation by assuming that cells were experiencing different conditions depending on their location with respect to the stimulation site.

**Results** The model describes Ca2+ metabolism after stimulation as follows: 1) Cells near the stimulus site are likely to conduct Ca2+ through plasma membrane stretch-sensitive Ca2+ channels and gap junctions. 2) The extracellular ligand and inositol 1,4,5-trisphosphate (IP3) diffusion through gap junctions mediate the signal in all locations of the monolayer, ligand concentration decreasing with distance. 3) The kinase activity targeted to IP3 receptor defines the sensitivity of the cell to the ligand. The model predicts suramin drug effects on P2Y2 receptors suggesting that suramin accelerates the phosphorylation rate of the receptors by enhancing their desensitization.

**Conclusion** Our model is the first mathematical model of Ca2+ signaling in ARPE-19 cells. The model enables the analysis of the Ca2+ signal propagation mechanisms, and predicts new pathways of suramin drug effects.

• 2475

**Scara5 involvement in retinal iron metabolism**

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NAVARRO M (2, 1), CARRETERO A (2, 1), RODRIGUEZ-BAEZA A (3),  
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**Purpose** Although transferrin is the principal source of iron in mammalian cells, recently, ferritin was proposed as a new iron transporter protein. Serum ferritin is composed mostly, but not exclusively, of L-ferritin, and in the presence of even a single H-ferritin chain, up to 4500 atoms of iron can be incorporated into one ferritin molecule. As each transferrin molecule only binds to two iron atoms, serum ferritin can be regarded as a potential deliverer of a considerable large amount of iron to tissues. Iron implication in oxidative damage has become clear, and it is well known that iron accumulation is associated in the retina with several retinopathies.

**Methods** Scara5 and L-ferritin expression in the retina were studied by means of laser confocal microscopy, rt-PCR and Western Blotting in ICR adult mice and in a murine model of retinopathy. Horse spleen ferritin was intravenously injected in healthy ICR adult mice and alteration in iron handling proteins expression was analyzed.

**Results** Scara5 receptors, that specifically bind to L-ferritin, were found in retinal endothelial and perivascular cells. Intravenous injected ferritin crossed the blood-retinal barrier (BRB), through Scara5 receptor binding, and accumulated in these cells, suggesting that serum ferritin, mostly composed by L-ferritin, can be transported across the BRB into the retinal parenchyma. During retinopathy, alterations in L-ferritin levels and Scara5 expression were evaluated.

**Conclusion** Serum ferritin uptake could represent a new pathway of iron delivery to the retina and points out perivascular cells as a key element in retinal iron traffic and during retinal iron accumulation associated with some retinopathies.

• 2476 / T008

**Glucocorticoid modulation of agonist induced microvascular endothelial permeability**

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**Purpose** Conditions such as diabetic retinopathy and neural inflammation involve microvascular barrier breakdown leading to leakage, tissue oedema and immune cell influx. Glucocorticoids(GCs) have been shown to improve the barrier function of the vasculature in retina and brain. It has been proposed that GCs function through tightening of specialized junctions between endothelial cells (ECs) and reduce paracellular permeability. We investigated the effects of hydrocortisone(HC),dexamethasone(DEX),triamcinolone(TA) and a selective glucocorticoid receptor agonist(SEGRA) on microvascular endothelial permeability induced by vascular endothelial growth factor(VEGF),lysophosphatidic acid(LPA) and histamine(Hist), all of which are proposed to be involved in diabetic and inflammatory neurovascular pathologies.

**Methods** In vitro studies were undertaken in cultures of primary rat brain and retinal microvascular ECs, as well as a novel immortalised rat retinal EC line, PT2. Junctional protein characterisation was performed by indirect immunocytochemistry and confocal microscopy. Permeability was measured by macromolecular flux assays.

**Results** Primary retinal and cerebral EC cultures exhibited an exquisite apico-basal polarity in their response to vasoactive compounds, maintained high barrier properties and sophisticated junctional protein complement. Passaged or immortalised EC had lost most of these features. HC and DEX were effective in suppressing VEGF-, LPA- and Hist-induced permeability. TA results were similar, except Hist-induced permeability was insensitive to TA. SEGRA was ineffective in preserving microvascular barrier function.

**Conclusion** The effectiveness of GCs depends on the pathology involved in particular the vasoactive substance in play.

• 2477 / T001

**Comparative gene expression analysis of corneal stroma mesenchymal stem cell-like cells, limbal epithelial stem cells and bone marrow-derived mesenchymal stem cells**

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**Purpose** Purpose Stem cells in the central part of the cornea serve an important regenerative and homeostatic function. We aimed to describe the genetic fingerprint of these cells and compare that to limbal epithelial stem cells (LESC) and bone marrow-derived MSCs (bmMSCs).

**Methods** Corneal tissue and bmMSCs were harvested from cadavers and healthy donors, respectively (according to the Guidelines of the Helsinki Declaration) and cultured ex vivo. MSC-specific cell surface markers- and genome-wide microarray analysis were performed using FACS and Affymetrix GeneChip Human Gene 1.0 ST Array (~23,000 gene transcripts).

**Results** Genes related to stemness (228), differentiation and lineage (220), cell cycle (108) and HOX, SOCS, Notch signaling (218) were collected into functional groups and clustered hierarchically: 45 genes were found to be specific for corneal stroma (CS)-MSCs, 62 for LESCs and 9 for bmMSCs. The hierarchical clustering clearly separated the CS-MSCs from the LESCs and bmMSCs, but formed a higher cluster with the later. The top 10 genes related to the differences were VCAM1, FNDC1, MFAP5, SFRP2, IGF2, MMP, ITGA2, COLEC12, SEMA3A and MGARP. Number of molecules functioning in cellular movement (381), cellular growth and proliferation (408), development (370) and cellular development (360) were found with top biological functions in CSMSCs compared to LESCs or bmMSCs ( $p < 0.001$ ).

**Conclusion** Our data show clear distinction between the studied stem cells based upon their gene expression patterns and strengthen the hypothesis that CSMSCs are derived from bmMSCs and not from LESCs.

**Industry-sponsored symposium 2: Micronutrition: Part of the retinal specialists' armamentarium ?**

• 2541

**Micronutrition; Opinion and use among European Ophthalmologists**

*ASLAM T  
Manchester*

**ABSTRACT NOT PROVIDED**

• 2542

**New evidence on nutritional supplements**

*CREUZOT C  
Dijon*

**ABSTRACT NOT PROVIDED**

• 2543

**How to go further? From fundamental research to clinical practice**

*DELMAS D, LAYANA A  
Dijon*

**ABSTRACT NOT PROVIDED**

**• 2621****The neuroscience of glaucoma in relation to the possibility for neuroprotection**

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Various theories exist to explain how glaucoma is initiated to result in the differential rate of retinal ganglion cell death. We suggest that initially the quality of the blood supply in the optic nerve head region is affected to cause a type of ischemia. This causes an alteration in ganglion cell mitochondrial homeostasis and an activation of astrocytes and other glia in the optic nerve head. Thereafter, as the disease progresses substances released from activated glial cells and also blue light reaching the retina act synergistically to cause the death of specific ganglion cells at different times. We therefore propose that the repertoire of receptors and number of mitochondria in individual ganglion cells relate to their time of death after glaucoma is initiated. These ideas will be presented, as they suggest that the causes and mechanisms for individual ganglion cells dying in glaucoma vary. Such a theory implies that substances with a single mode of action is unlikely to be sufficient for effective clinical neuroprotection but that this might be achievable using substances with multiple modes of action or a suitable cocktail mixture of products.

## SIS: Vitreoretinal interface disorders

### • 2611

#### Normal and abnormal vitreoretinal interface conditions

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Pathologic changes at the vitreoretinal interface and vitreomacular traction, lead to the phenotypic lesions as epiretinal membranes (ERMs), idiopathic macular holes, vitreomacular traction syndrome (VMTS) and myopic foveoschisis. Remnants of vitreous collagen fibers in the presence of clinically evident posterior vitreous detachment (PVD) and/or cellular proliferations of fibrous astrocytes or glial cells growing outward from the retina to the inner retinal surface through the internal limiting membrane (ILM), in the presence of growth factors as laminin, fibronectin, may contribute to fibrocellular proliferation at the retinal surface. Most studies of idiopathic or diabetic macular holes related epiretinal membranes, described the presence of fibrocytes or myofibroblasts and more recent studies confirmed these observations using antibodies against alpha smooth muscle actine (a-SMA). Based on the mechanistic association between a-SMA expression and tractional force generation, there is little doubt that these cells represented the source of traction during the evolution of those pathologies.

### • 2612

#### Assessment and classification of vitreoretinal interface disorders

*TADAYONIR*

*Paris*

#### ABSTRACT NOT PROVIDED

### • 2613

#### Natural history of vitreoretinal interface disorders

*POURNARAS JA*

*Jules Gonin Eye Hospital, Lausanne*

Natural History of main vitreoretinal interface disorders as epiretinal membranes, macular holes, vitreomacular traction syndrome (VMTS) and myopic foveoschisis will be discussed.

### • 2614

#### Medical management of vitreoretinal interface disorders

*LE MER Y*

*Fondation Ophtalmologique A. de Rothschild, Paris*

Year 2013 will be a cornerstone in the history of therapeutic vitreolysis with the European approval of the ocriplasmin in March, the first commercially available product to achieve in some selected cases a separation of the vitreo-retinal interface. Since the publication in 1993 of the first vitreous detachments in rabbit eyes, obtained by intravitreal injection of plasmin, the road has been opened to several researches looking for the best candidate to produce this detachment in human eyes. The published data of the use of ocriplasmin to date are still too fragmentary to give a precise idea of what could be the best indications to perform an intravitreal injection of drug instead of a classical vitrectomy. Aside the symptomatic vitreo-macular traction syndrome and small idiopathic macular holes, some other disorders in which an abnormal vitreoretinal adhesion seems to play a role are currently evaluated, such as the early stages of macular degeneration and diabetic macular edemas. We'll present the last available data, focusing beyond the pure scientific results, on the possible consequences on our everyday surgical practice and the clinical decision tree in vitreo-retinal disorders.

#### Commercial interest

**• 2615****Surgical management of vitreoretinal interface disorders***WOLFENBERGER T**Jules Gonin Eye Hospital, Lausanne*

Purpose: To assess safety and efficacy of novel surgical therapies for vitreomacular interface disordersBackground: Vitreomacular interface disorders are characterised by a combination of mechanical and biochemical interactions between the posterior hyaloid and the internal limiting membrane and the retinal surface in general. Pathologies that are amenable to surgical treatment include primarily epiretinal membranes and macular holes, but other less common maculopathies have been come to the forefront recentlyResults: This presentation will summarise the latest developments in the surgical therapy of vitreomacular interface disorders by evaluating the most recent data on advances in diagnostic tools for preoperative decision making, surgical technology and pharmacological combination therapiesConclusion: Novel surgical techniques are constantly increasing the therapeutic success while reducing tissue irritation during surgery. New diagnostic approaches including high-resolution OCT can help the decision making and to predict visual acuity recovery after vitreomacular interface surgery.

**• 2616****Case study***POURNARASJA**Jules Gonin Eye Hospital, Lausanne*

Case Study will be proposed and therapeutical options will be discussed with the panel.

## SIS: New angles in glaucoma- neuroprotection

### • 2621

#### The potential of regenerative strategies in glaucoma

CORDEIRO M  
London

Scientists have for years been investigating why a salamander can regenerate cut limbs and nerves. In an attempt to look at possible explanations, whole eye transplants were performed in these in animals as far back as almost a century ago. Many groups around the world have identified however, several barriers to successful nerve regeneration, including: maintaining the viability of damaged neurons; sufficient re-elongation of surviving neuronal fibres; penetration of growing nerve fibres through scar tissue; the overcoming of growth inhibitory signals and finally the guidance of nerve fibres to the original target to reform "functional connections". In glaucoma and optic nerve damage, because RGC axons have to project long distances to the lateral geniculate nucleus in the thalamus, up to now, the "rewiring in the brain" has proved to be an insurmountable obstacle. However, there has been very recent significant progress in the fields of neuroprotection and RGC survival, scar and growth inhibitor modulation, guidance signal and *in vivo* assessment of structural effects, making optic nerve regeneration and repair a real possibility in the future.

### • 2622

#### The potential role of endothelin in glaucoma

CHAUHAN B  
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Endothelin is a potent vasoactive peptide occurring in three isotypes (ET-1, ET-2 and ET-3). Endothelin via its two main receptors ETA and ETB is responsible for a variety of physiological functions primarily blood flow control. Recent evidence from both human and experimental optic neuropathies shows involvement of endothelin and upregulation of its receptors (principally ETB). Experimental studies have shown that chronic ET-1 administration to the optic nerve immediately behind the globe causes neuronal damage, activation of astrocytes, the major glial cell in the anterior optic nerve and upregulation of ETB receptor. This review outlines the ubiquitous role of endothelin and its potential involvement in glaucoma.

### • 2623

#### The role of low-grade inflammation in glaucoma

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Glaucoma has been associated with low-grade inflammation. The exact role of inflammatory processes in the pathophysiology of various types of glaucoma is, however, unclear. In addition, few *in vivo* studies have provided direct evidence for inflammatory processes in glaucoma. Disturbed auto regulation has been shown to increase the levels of Endothelin-1 (ET-1) and/or nitric oxide (NO) thereby causing inflammation by either inducing ischemia or oxidative stress. Evidence has furthermore revealed a diminished Müller cell ability to remove excess glutamate in the synaptic cleft in response to vascular dysregulation. This in turn causes glutamate excitotoxicity with a hyperstimulation of the NMDA receptor. Activation of NMDA-receptors has been shown to induce TNF- $\alpha$  production in the Müller cells, indirectly causing RGC death through TNF- $\alpha$  stimulation of TNF- $\alpha$  receptor 1 on RGC. Specifically, this aspect of RGC death rise the possible involvement of low-grade inflammation as an important factor in the metabolic and excitotoxic intradependance between Müller cells and RGC. The current knowledge of low-grade inflammatory changes in glaucoma and ideas on possible future targets for pharmaceutical intervention in glaucoma will be discussed

**• 2631****Step by step limbal stem cell transplantation techniques***GICQUEL J (1), DUA HS (2)**(1) Poitiers**(2) Nottingham***ABSTRACT NOT PROVIDED****• 2632****Indications and practical use of corneal crosslinking***MENCUCCI R**Eye Clinic University of Florence, Italy*

Indications and practical use of corneal crosslinking

**SUMMARY** Corneal crosslinking with riboflavin and UVA irradiation is a recent and promising approach to increase the mechanical and biochemical stability of the stromal tissue in keratoconus. Nevertheless there are still unsolved issues about this procedure, such as the more effective and tolerated imbibition time, irradiation potency and riboflavin solution composition; new procedure has been proposed such as transepithelial crosslinking, accelerated crosslinking, imbibition with iontophoresis. Indication, contraindication, potential risk and benefits of these procedures will be reviewed.

**• 2633****Modern indications and limitations of amniotic membrane transplantation***YELING A**Birmingham***ABSTRACT NOT PROVIDED****• 2634****Bloodless and painless pterygium surgery***GICQUEL JJ**Poitiers*

Pterygium is the most common surgically managed ocular surface disorder. The primary Goals are to prevent recurrences in advanced cases and get a proper esthetic result in younger patients with less symptomatic cases. There has been 1 article every month for the last 4 decades on the subject... Bare sclera technique should be avoided at all costs, because it involves too many recurrences. Hence will discuss other techniques, such as conjonctivo-limbalautograft (sutured or glued), conjunctival graft, amniotic membrane and the use of anti-mitotic drugs.

## Free papers PO 2/2: Diagnostic tools in eyelid and orbital pathology

### • 2641

#### **Agreement between in vivo confocal scanning laser microscopy with a handheld microscope and histology in eyelid and conjunctival tumours**

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 (2) Department of Ophthalmology, University Hospital, Saint-Etienne  
 (3) Department of Dermatology, University Hospital, Saint-Etienne  
 (4) Department of Pathology, University Hospital, Saint-Etienne  
 (5) Institut Universitaire de France, Bd St Michel, Paris

**Purpose** By providing non-invasive pseudo histology, in vivo confocal microscopy (IVCM) has been proven efficient in the diagnosis and treatment management of numerous diseases in dermatology and in ophthalmology for the ocular surface. Although it is only complementary to classical histology it allows quicker decisions. At present, both ophthalmology IVCM available are nevertheless not fully convenient for the exploration of the whole conjunctival surface nor for the entire eyelids, because of their bulk and their limited mobility. Aim: to study the reliability of a dermatological handheld IVCM for eyelid and conjunctival tumours

**Methods** Thirty-one tumours were analysed using the handheld dermatological IVCM Vivascope 3000 (Lucid Inc, NY, MAVIG GmbH, Germany) equipped with 830nm class IB laser. IVCM diagnosis was made by an ophthalmologist and a dermatologist with a standardized reading grid. Whenever surgery was necessary, tumours were analysed by pathologists blind to the IVCM diagnosis. Agreement between both diagnoses was determined by an independent observer, who calculated sensitivity, specificity, predictive pos value, and predictive neg value

**Results** All tumours were accessible. Overall scores were Se=1, Sp=0.45, PPV=0.75, PNV=1, for conjunctiva Se=1, Sp=0.4, PPV=0.571, PNV=1, for eyelids Se=1, Sp=1, PPV=1, PNV=1, and for eyelid margin Se=1, Sp=0.67, PPV=0.75, PNV=1

**Conclusion** The handheld IVCM is perfectly adapted to the exploration of the entire conjunctival and eyelid system. Grant: GIRCI Rhone Alpes Auvergne 2012No financial interest for all authors

### • 2643 / S086

#### **A novel imaging approach to periocular basal cell carcinoma: In vivo optical coherence tomography and histological correlates**

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**Purpose** Optical coherence tomography (OCT) is a non-invasive imaging method widely used in ophthalmology. Recent developments have produced OCT devices for imaging the skin. The purpose of this study was to assess preoperative OCT of periocular basal cell carcinoma (BCC) and to correlate optical biopsies with histological features.

**Methods** Consecutive patients with periocular BCC were prospectively investigated with VivoSight OCT (Michelson Ltd) prior to surgical excision. OCT images were correlated to haematoxylin and eosin stained histology sections with regard to: epidermal changes, intralesional and perilesional features.

**Results** A total of 15 patients with periocular BCC were recruited. Epidermal changes over BCC lesions represented important landmarks for topographic correlation and consisted in epidermal thinning and hyper-reflective signals from surface ulceration and crusting. Intralesional OCT features of BCC included: hyporeflective lobular patterns corresponding to BCC nests with high cellularity, hyporeflective cystic spaces of liquefactive necrosis and hyper-reflective margins corresponding to collagen compression. Perilesional OCT features of BCC included: hyporeflective spaces corresponding to fluid clefts, hyper-reflective bands of perilesional granulomatous inflammation secondary to tissue biopsy and hyporeflective lumens of blood vessels and hair follicles

**Conclusion** This study demonstrated a high correlation between OCT images and histological features. Further studies are necessary to investigate novel potential applications of in vivo OCT for BCC such as non-invasive diagnosis, intraoperative OCT-guided tumour excision and postoperative detection of recurrences.

### • 2642

#### **Mohs surgery of eyelid tumours assisted by ex vivo confocal microscopy and a new “open book” method for tissue preparation**

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**Purpose** The micrographic Mohs surgery, that allows a complete and tissue-economic resection of skin tumours, is particularly adapted to eyelid margin tumours (EMT) surgery. However, being time consuming for pathologists it is not used in routine. Aim: to expose a fast and reproducible method of Mohs surgery for EMT associating ex vivo confocal microscopy (EVCM) and an original tissue preparation named “open-book”

**Methods** EMT were surgically resected, let unfixed and unstained, immediately prepared with the “open book” technique and analysed with EVCM (vivascope 2500, Lucid Inc, NY and MAVIGH GmbH, Germany). The “open book” technique consisted of splitting the eyelid in two lamellae maintained by a hinge situated at the eyelid margin and flat-mounting it between two glass slides in order to allow acquisition of both cutaneous and conjunctival sides in the same EVCM scans. The whole sample volume was scanned at the 3 wavelengths available (488, 658 and 785 nm) and the mosaics was automatically reconstituted by the built-in software. Tumours were then immediately fixed in 4% PFA and a classical pathologic analysis was performed. Agreement between tumour limits found in EVCM analysis and in classical pathology was calculated with weighted kappa coefficient

**Results** In all cases, the skin and conjunctival tumours limits determined by EVCM were comparable to classical pathology

**Conclusion** ECVM combined with original “open book” tissue preparation could take a place in routine of eyelid tumour surgery. Grant: GIRCI jeune chercheur Rhone Alpes Auvergne 2013No financial interest for all authors

### • 2644 / S088

#### **Unique morphology of the human orbit among the Hominoidea**

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 (2) Hear, Nose and Throat, Caen  
 (3) Biostatistics, Caen

**Purpose** Like many mammal predators, Humans have frontal (forward-facing) orbits. This design allows a large overlap of monocular visual fields with good stereoscopic vision but is considered to harm lateral space perception. In Humans, on average, temporal visual field extends 95° in primary position of gaze but 128° with eye abduction. Which anatomical peculiarity may allow such a visual field expansion?

**Methods** Comparative orbit osteology study in 100 human skulls and 120 Apes' skulls (30 gibbons; 30 orang-utans; 30 gorillas; 30 chimpanzees and bonobos). Orbit width and height were recorded. Using a protractor and laser levels two orbit angles were recorded: “convergence angle” (the lower this angle, the more frontal the inner orbital rim orientation) and “opening angle” (the higher this angle, the more backward the temporal orbital rim position).

**Results** The largest orbit width/height orbit ratio is 1.19, in Humans ( $p < 0.001$ ). Humans have a higher “convergence angle” (98.1°) than all Apes except gibbons (99.2°;  $p > 0.05$ ). Humans have by far the largest “opening angle” (107.1°;  $p < 0.001$ ) and the largest difference between “opening angle” and “convergence angle” (9°;  $p < 0.001$ ).

**Conclusion** The largest orbit width/height ratio found in Humans suggests a design that favours lateral vision. More specifically, human orbital rim is unique in that, while frontal, it has by far the most backward temporal orbital rim. This peculiarity - likely and adaptation to terrestrial life with upright bipedal locomotion - allows both good stereoscopic vision and large temporal visual field extent through eye motion.

• 2645 / S087

**Strabismus and diplopia revealing rhabdomyosarcoma in a 7-year-old girl**

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**Purpose** This case report presents the diagnosis and management of seven-years-old girl with persistant diplopia revealing orbital rhabdomyosarcoma ,a rare and malign type of tumor among children. Few cases have been reported.The clinical presentation, complementary exams, and treatment for this condition are reviewed.

**Methods** A 7-year-old girl was referred to our department for persistant diplopia ,and unilateral exophthalmia on her left eye that had been lasting for 5 months.The orthoptic examination showed no visual acuity loss, vicious head tilt bent on the left side, exotropia with quick restitution, limitation of abduction and elevation on left eye. The Lancaster test confirmed the limitation.The eye fundus examination revealed signs of extrinsic compression.

**Results** Magnetic resonance imaging, and ocular and orbital echography showed an intra conical tumor of 18x21millimeters, displacing the ocular nerve and invading the superior rectus muscle. Tumor biopsy revealed undifferentiated rhabdomyosarcoma. Metastatic work-up was negative and included thoracic and abdominal CT, bone scintigraphy and bone marrow biopsy. The treatment consisted in chemotherapy and permitted tumor volume regression of 85%. The patient has successfully manage with muscle surgery, not on the cicatrical site but on the other eye.

**Conclusion** Ocular rhabdomyosarcoma is a rare tumor among children, but the most frequent malign orbital tumor, with possible secondary localisation. Early diagnosis and early treatment are determinant for the prognosis and the survival rate of these patients. This case report shows all the utility of strabismological examination to detect the first signs of the condition, even without any symptoms relative to visual acuity.

• 2646

**Non graves orbital inflammations: Rationale for a diagnostic approach**

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(1) Ophthalmology, Nice  
(2) Ophthalmology, Limoges

**Purpose** To propose a diagnostic approach for clinicians who manage a patient with non Graves' orbital inflammation.

**Methods** A review of the diagnostic process for sixty one patients with diagnostic of orbital inflammation between january 2002 and january 2013 at Nice and Limoges University Hospitals.

**Results** Sixty one patients presenting with orbital inflammation were included. More common presentations were orbital fat inflammation (60%), myositis (26%) and lacrimal gland enlargement (18%). In few cases, optic nerve and sclera were involved (respectively 9,8 and 3,2%). The final diagnostic was idiopathic orbital inflammation (25 patients), specific orbital inflammation (17 patients) and orbital lymphoma (19 patients). Thirty six patients had biopsy first and 25 patients did not, in first, but only after failure to the initial therapy.Our results showed that doing biopsy in the initial management of orbital inflammation provides a more great number of specific diagnosis than doing treatment tests directly after blood tests or CT scans ( p>0,01).

**Conclusion** The diagnostic process of orbital inflammation must include blood testing, imaging of the orbit and sinuses, and biopsy in first intention as far as possible.

• 2647

**Repair of orbital implants exposures using muller muscle flaps**

ROBERT PY, DELMAS J, ADENIS JP  
CHU Dupuytren, Limoges

**Purpose** To evaluate a two-stage technique of Müller muscle and conjunctival flap for treatment of orbital implant exposure defects.

**Methods** In this retrospective study, 19 patients with acquired anophthalmic socket presenting an exposure of orbital implant were included, between March 1999 and September 2012.

**Results** 13 women, 6 males were managed with this two-stage procedure. Medium age was 57.5 years (9 to 90). The average follow-up was 41 months (3 to 163). 16 patients underwent an evisceration and 3 patients an enucleation. All of them were implanted primarily with hydroxyapatite (HA) implants, except one of them who had an acrylic implant. Orbital implant exposure was note at an average of 94.4 months (2 to 240) after implantation. The average period between the first-stage (Müller muscle flap) and the second-stage (section of the flap) was 39.9 days.Success rate was 68.4% (13 patients) and the failure rate was 31.6% (6 patients) who required second surgery (one or two-stage implant ablation followed by dermis fat graft, or temporalis fascia graft). Predisposing factors for exposure were noticed among patients with failed Müller muscle flaps: 2 underwent an enucleation for melanoma followed by radiation therapy, 1 patient had an acrylic non porous implant. 1 patient presented an early exposure probably due to suture under stress. But 2 others patients did not present predisposing factors for exposure. Once the exposure was treated, all of the patients were fitted with a prosthetic device successfully.

**Conclusion** Müller muscle flap 2-stage procedure is a reliable technique, with a success rate superior to 68%, allowing preservation of the implant and successful equipment with prosthetic devices.

## Free papers IM 1/2: Ocular infections "pole to pole": new perspectives

### • 2651

#### Inhibition of the DNA damage response blocks herpes simplex virus infection in corneal epithelium

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**Purpose** Herpes simplex keratitis (HSK) is the leading cause of cornea-derived and infection-associated blindness in the developed world. Many HSK cases are refractory to treatment with available drugs, leading to permanent corneal pathology. In addition, drug-resistant viral strains are beginning to emerge and may present a major clinical problem in the future. Thus, our study focused on identifying and targeting critical virus-host interactions to suppress herpes simplex virus (HSV) infection in corneal epithelium. Specifically, we investigated the role of ataxia telangiectasia mutated (ATM), a key protein in the mammalian DNA damage response pathway, in the molecular pathogenesis of HSK.

**Methods** We utilized three experimental models in our studies. The *in vitro* model (human corneal epithelial and keratinocyte cell lines) was used to dissect the molecular mechanisms of HSV-ATM interactions. The *ex vivo* model (human and rabbit explanted corneas) was used to validate our tissue culture findings. And the *in vivo* model (young C57BL/6 mice) was used to test the antiviral potential of ATM inhibition in a physiologically-relevant context of ocular infection.

**Results** ATM inhibition reduces HSV infection in corneal epithelial cells by blocking viral genome replication. Importantly, this inhibitory effect was observed not only with wild type HSV-1, but also with two different drug-resistant strains. The antiviral effects of Acyclovir and ATM inhibition were additive. We have also made progress into elucidating the molecular mechanisms underlying this phenomenon.

**Conclusion** We have shown that ATM is a new therapeutic target for the treatment of herpes simplex keratitis. ATM inhibitors could be used alone or as adjuvant therapy to combat HSK.

### • 2652

#### Small molecule design strategy to overcome antibiotic resistance

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(2) Ophthalmology, Tampere

(3) Duke-NUS SRP NBD, Singapore

**Purpose** Although antimicrobial peptides have been shown to avert resistance, most small molecule antibiotics do not share that characteristic. Antimicrobial peptides though are often less stable environmentally and have difficult delivery problems. However, small molecules are much easier to develop from a pharmaceutical perspective. This study has combined the properties of antimicrobial peptides into a small molecule platform.

**Methods** Using our design platform which consists of microbiology, biophysical (including NMR), and animal data we have used the molecular backbone of a xanthone, alpha-mangostin which is extracted from the pericarp of the SE Asian fruit, mangosteen. The xanthone is a heterocyclic planar molecule with sites at carbons 3 and 6 modifiable with amino acids or other functional groups. Stable, water soluble compounds have been developed whose activities are tested *in vitro* in microbiological assays as well as in mouse models of corneal infection. These molecules have been tested in MIC, time kill studies and in simulations of resistance.

**Results** The results show that the antimicrobial activities of the cationic xanthone derivatives can be generally predicted based on the pKa values of the corresponding amines. We have identified AM-0016 (3b) as the most potent compound in the series with potent antimicrobial activity with MIC values of 0.095-0.39 ( $\mu$ g/mL) against Gram-positive bacteria including MRSA, improved selectivity up to 200, rapid time-kill in 10-30mins,

**Conclusion** A series of novel antimicrobials have been designed and prepared by cationic modifications of  $\alpha$ -mangostin, a natural xanthones with a planar hydrophobic core, to yield an amphiphilic structure which improves selectivity for bacterial membranes through the hydrophobic-water interface perturbation.

### • 2653

#### Pharmaceutical failure mechanism of the worldwide ReNu with MoistureLoc-related fusarium keratitis epidemic of 2004-2006

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**Purpose** Between 2004-2006, a worldwide epidemic of Fusarium keratitis occurred, traced to Bausch & Lomb's ReNu with MoistureLoc contact lens solution (RML), containing the antimicrobial agent, alexidine dihydrochloride. Our previous studies indicated that heating RML ( $>42^{\circ}\text{C}$  and  $\leq 56^{\circ}\text{C}$ ) in its plastic (but not a glass) bottle resulted in decreased anti-Fusarium capability. The present study was undertaken to determine the exact mechanism of this antimicrobial failure.

**Methods** We investigated if: microbiologically and/or analytically (using Raman spectroscopy), an alexidine-neutralizing leachate emanates from heated ReNu bottles; the alexidine concentration (measured by liquid chromatography coupled with tandem mass spectroscopy) changes when stored in heated ReNu bottles; alexidine permeates into ReNu bottle walls and can be detected by Fourier transform infrared spectroscopy; non-alexidine antimicrobials fail in heated ReNu bottles; and/or, alexidine fails in heated non-ReNu bottles.

**Results** No leachates were identified microbiologically or analytically. The alexidine concentration was 2.8 times greater in room temperature- (vs.  $56^{\circ}\text{C}$ -) stored ReNu bottles. Alexidine deposited into the ReNu bottle wall 3.1 times greater in the heated (vs. room temperature-stored) container. Non-alexidine antimicrobials retain their anti-Fusarium capability after being heated in ReNu bottles and alexidine heated in non-ReNu bottles did not lose its anti-Fusarium activity.

**Conclusion** Alexidine permeates into the walls of heated ReNu plastic bottles, diminishing its concentration in solution and allowing Fusarium growth. This appears to be the mechanism of the RML-related Fusarium keratitis epidemic.

### • 2654

#### Recurrence of toxoplasmic retinochoroiditis: Analysis of cases proven by initial analysis of aqueous humor

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**Purpose** Factors explaining recurrences of toxoplasmic retinochoroiditis (TRC) and their frequency are not understood. The aim of this study is to analyze their recurrence patterns, and search for an association with their clinical and biological features.

**Methods** 40 consecutive cases of TRC with a positive aqueous humour (AH) tap for toxoplasmosis were retrospectively included. Inclusion criteria were: Goldman-Witmer coefficient (GWC)>2.0, positive immunoblot (IB) or positive PCR for Toxoplasma gondii. Further episodes, their characteristics, disease-free intervals, and treatments were collected. We performed a case-control analysis of patients with and without recurrences during follow-up.

**Results** Mean age was 41.5 years. 10% of patients were immunodeficient (n=4). Mean follow-up was 7.3 years (5.8-10.3). Forty-three percent of patients (n=17) presented at least one recurrence, with a mean of 1.7 episodes (1-4) per patient. Mean interval between episodes was 27 months (3-73). For patients experiencing one recurrence or more, initial AH analysis showed a lower GWC, less additional bands on IB, and less positive PCRs, even though these trends did not reach statistical significance. Clinically, these patients had broader lesions, in more peripheral locations, and more intense vitritis than those with no recurrences. For the initial episode, durations of antibiotic and anti-inflammatory treatments were superior in the recurrence group. Sex, ethnicity, or immune status were not associated with a change in recurrence risk.

**Conclusion** TRC recurrences seem to be influenced by clinical and biological features at baseline.

• 2655

**Reduced Th17 type inflammation associated with enhanced Th1, Th2 and Treg responses in a model of reactivation of congenital ocular toxoplasmosis**

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**Purpose** Ocular toxoplasmosis (OT) is a major cause of blindness in the world. Ocular involvement is frequently seen following congenital infection. Many of these infections are quiescent but pose a life-time risk of reactivation. We previously developed a Swiss-Webster outbred mouse model for congenital toxoplasmosis by neonatal injection of *Toxoplasma gondii* cysts. We also used a mouse model of direct intraocular infection to show a deleterious local Th17 type response upon primary infection. However, little is known about the physiopathology of reactivation.

**Methods** In the present study, we combined our two models to study reinfection into neonatally infected mice, in comparison with a primary ocular infection. Intraocular immunological determinants were studied using both BioPlex proteomic assays in aqueous humor and RT-PCR for crucial transcription factors.

**Results** We observed diminished Th17 type reaction in reinfection, compared to primary infection. In contrast, Th2 and T regulatory responses were enhanced. Interestingly, this was also true for Th1 responses, which was paralleled by a better parasite control. We observed a similar protective immune reaction pattern in the eye upon reinfection with the virulent RH strain, with the notable exception of IFN- $\gamma$ .

**Conclusion** In summary, our results show a less pathogenic but more effective anti-parasite pattern during reinfection.

• 2656

**Anatomical and visual outcome after pars plana vitrectomy in acute postcataract endophthalmitis**

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(4) *Department of Ophthalmology, University Hospital, Dijon*

**Purpose** To report visual and anatomical outcome of patients vitrectomized for acute post bacterial endophthalmitis

**Methods** 123 patients with acute postcataract endophthalmitis and consecutively treated by pars plana vitrectomy (PPV) were included in four academic hospitals (French Institutional Endophthalmitis Study (FRIENDS) group).

**Results** At 6 month follow-up, 49 patients (40%) had a visual acuity (VA) greater than or equal to 20/40, and 83 patients (68%) had VA greater than or equal to 20/200. Baseline factors associated with final VA  $\geq$ 20/40 were a younger age (Odds ratio, OR= 1.05 (1.05-1.09), p=0.006), presence of fundus visibility (OR=7 (1.4-34.6), p=0.007), and absence of cataract surgery complications (OR=15.3 (1.9-32), p=0.001), corneal edema (OR= 2.3 (1.1-4.9), p= 0.02), hypopion (OR= 2.6 (1.1-6.6), p=0.04), or of virulent bacteria (OR=3.1 (1.4-6.7), p=0.005). At the time of PPV, absence of vasculitis at the posterior pole at the time of PPV (OR= 7 (1.4-34.6), p=0.02) and a lower duration of PPV (OR=1.02 (1.01-1.04), p=0.02) were significantly associated with final VA  $\geq$ 20/40. Risk factors of RD were diabetes (OR = 4.7 (1.4-15.4), p=0.01), and visualization of retinal vasculitis on the posterior pole (OR = 3.8 (1.1-13.9), p= 0.03) at the time of PPV.

**Conclusion** PPV allowed to be beneficial in a majority of the patients. Baseline inflammatory signs and bacterial virulence are the main prognostic visual factors. RD remains the major complication but final anatomical and visual outcome is still poor, despite updated vitreoretinal techniques

## SIS: Mitochondrial dysfunction in optic neuropathies: from disease mechanisms to therapeutic strategies

### • 2661

#### **Genetic analyses of autosomal optic neuropathies reveal novel physiopathological pathways involved in optic nerve degeneration**

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Autosomal Optic Neuropathies (AON) are rare mitochondrial diseases affecting the retinal ganglion cells that form the optic nerve and transduce the visual informations from the retina to the brain. They are heterogeneous inherited diseases with dominant – the most abundant - and recessive forms, for which only three genes have so far been identified, OPA1, OPA3 and OPA7. Together, mutations in these genes account for less than 50% of patients with an ascertained clinical diagnosis, OPA1 being by far the most frequent gene involved. To gain insight in the genetic diagnosis of AON, we performed whole exome sequencing on dominant and recessive families, for which we excluded mutation in OPA1, 3 and 7. Results identified some novel genes, all encoding mitochondrial matrix proteins. Importantly none encodes protein embedded in the inner mitochondrial membrane, as are OPA1, 3 and 7, but all have a function related to the lipid and protein composition of this membrane. Thus these results put emphasis on novel pathophysiological mechanisms involved in retinal ganglion cells degeneration, again pointing on the absolute requirement to maintain the inner mitochondrial integrity to preserve optic nerve function and survival.

### • 2662

#### **OPA1 in mitochondrial quality control and its implications for RGC degeneration**

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Mitochondria are vulnerable to damages of their proteins, lipids, and mtDNA by various stress factors and mitochondria either recycle dysfunctional mitochondria via the mitochondrial autophagy pathway, arrange for the recycling of the complete host cell by apoptosis, or continue to function normally and supply the host with energy and metabolites. This is called mitochondrial quality control and it is crucial for all neurodegenerative diseases, including the most prominent ones, Alzheimer's disease and Parkinson's disease. Mitochondrial quality control is facilitated by mitochondrial network dynamics – continuous fission and fusion of mitochondria. Mitochondrial fission is accomplished by DRP1, MFN1 and MFN2 on the mitochondrial outer membrane, and OPA1 on the mitochondrial inner membrane are necessary for mitochondrial fusion. Mitochondrial network dynamics are regulated in highly sophisticated ways by post-translational modifications and protein processing. I would like to present selected aspects of mitochondrial quality control using OPA1 as an example and discuss their implication on Dominant Optic Atrophy, a slowly progressive optic neuropathy with juvenile onset caused by mutations in the OPA1 gene.

### • 2663

#### **Mitochondrial dynamics in mitochondrial optic neuropathies**

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OPA1 mutations are the commonest cause of dominantly inherited optic atrophy (DOA) and so-called DOA plus. The Opal protein is essential for normal mitochondrial fusion. In mouse DOA, autophagy (recycling of spent cellular components) is dysregulated in retinal ganglion cells (RGCs), and mitophagy (mitochondrial recycling) has been implicated. We validated and used novel ImageStream technology to quantitate mitophagy in primary cells. Co-localisation of mitochondria and autophagosomes was increased in fibroblasts in five patients from four families with severe OPA1 phenotypes indicating increased mitophagy. ImageStream also showed that basal mitophagy was increased when control cultures were depleted of Opal by siRNA. Western blotting confirmed increased basal autophagy and autophagic flux in the presence of activators. Increased mitochondrial fragmentation, mitophagy and failure of mitochondrial transport may together cause local depletion of mitochondria in critical regions of the retinal ganglion cells, such as axons or synapses. Fragmentation may also impair stress induced mitochondrial hyperfusion. Increased mitochondrial fragmentation, mislocalisation and mitophagy thus link low OPA1 to neurodegeneration.

### • 2664

#### **New concepts of treatment for LHON and other genetic mitochondrial optic neuropathies**

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In Leber's Hereditary Optic Neuropathy (LHON), complex I impairment can lead to subacute loss of vision and optic nerve atrophy, usually in young adulthood. This blockage of electron transfer produces some decrease in ATP production, but also a severe accumulation of reactive oxygen species (ROS) that is thought, at a critical threshold, to lead to RGC apoptosis. Co-enzyme Q10, a quinone, has the potential to reroute the spilled electron, however treatment with co-enzyme Q10 has been ineffective. Idebenone, a second generation quinone with better drug delivery characteristics, has demonstrated some efficacy in LHON (results presented in a companion presentation). EPI-743 is a third generation quinone that has other promising characteristics. We provided EPI-743 (300 mg, TID) in an open label trial to 12 patients with recent visual loss from LHON and carefully followed 23 eyes for 2-3 years (16 to 40 months). Seven of 23 eyes demonstrated improvements in visual acuity and 5/23 improvements in HVF. Thirteen of 23 eyes showed improvements or at least acute stabilization of vision with these outcome measures. Oddly, the time to recovery was very long (9 to 15 months).

*Commercial interest*

**• 2665****Raxone (idebenone) in LHON - An update***MEIER T**Santhera Pharmaceuticals, Liestal*

Leber's Hereditary Optic Neuropathy (LHON) is caused by mtDNA mutations affecting subunits of Complex I of the mitochondrial electron transport chain. Affected mutation carriers suffer from rapidly progressing and mostly irreversible bilateral vision loss. An increasing body of evidence indicates that idebenone has therapeutic potential for the treatment of LHON. Data from a randomized placebo-controlled study (RHODOS) and from a number of case reports and retrospective cohort studies demonstrate that patients with established vision loss may benefit from idebenone treatment and recover visual acuity. Early treatment start (e.g. within one year from onset of symptoms) appears to be associated with better outcome, which is supported by the cellular pathology of LHON. Data from a currently on-going named patient program (NPP), where LHON patients receive 900 mg/day Raxone® under routine clinical care, will be presented and an update on the regulatory status of Raxone® provided.

*Commercial interest*

**Joint Meeting: EEBA symposium: storage and processing of donor tissue before transplantation**

• 2671

**Hypothermic storage or organ culture?**

PONZIND

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Currently there are two storage practices for the cornea, both use liquids based on cell culture medium: the hypothermic storage at 2-6°C, and organ culture at 30-37°C. Hypothermic storage seems to offer donor tissues of good quality comparable to that obtained by organ culture, provided that the storage time is kept short. Indeed, according to the literature, the risk of primary graft failure increases significantly after storage longer than 7 days. Furthermore, corneas stored longer than 7 days display epithelial alterations that may hinder the surgical procedure or delay the full recovery of the graft. The organ culture storage method consists of a storage period in culture medium at 30-37°C, and a shorter de-swelling and transportation phase at 30-37°C and room temperature in the same medium supplemented with 4-8% dextran. Organ culture solutions contain fetal calf serum as a source of growth factors to limit endothelial cell loss. The serum must be obtained from prion-free countries. A storage period of 30 days can be achieved without significant loss of endothelial cells. The endothelium shows reparative phenomena during storage. Organ culture offers a longer storage time, a less restricted donor supply on beforehand, corneal endothelium with a better defined quality, and a pre-operative microbiologic control. Cultured corneas always display an epithelium made up of 2-3 layers of viable cells. The 30 days storage period allows an efficient use of valuable donor tissue: planning of operations is easier, allowing sufficient time for the allocation. The disadvantages of this method are the relative technical complexity and the need for qualified staff to perform tissue culture and selection of the corneas. The studies comparing the effect of the storage methods on outcome demonstrate similar graft survival and post-operative decline in endothelial cell density. Corneas stored by organ culture are at least comparable with those stored hypothermically for shorter periods.

• 2672

**Consequences of storage on the donor corneal tissue**

ARMITAGE J

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No storage method for biological materials preserves the full functional integrity of all cells in a given population. The loss of cells during storage of tissues for transplantation has to be accepted and the purpose of storage is to minimize both cell loss and maintain functional integrity. This is especially important for tissues where integrated cellular function is essential for normal, overall physiological function of the tissue. This is the case for cornea intended for transplants that require an intact and functioning corneal endothelial monolayer, such as for endothelial disease. The three main approaches to corneal preservation, viz., organ culture, hypothermic storage and cryopreservation, all pose their own unique challenges to cells and present a range of differing mechanisms of damage. Hypothermia relies on the suppression of metabolism by reduced temperature. Organ culture attempts to maintain metabolism. Cryopreservation completely suppresses all biochemical reactions and offers the prospect of indefinite storage. A better understanding of these mechanisms may lead to improvements in the quality of tissue after storage.

• 2673

**The fate of the corneal endothelium during storage: apoptosis/necrosis/survival**

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Cell death during corneal storage is, at least with current methods, inevitable. There are also concerns with a tissue such as cornea for the maintenance of cell-cell and cell-matrix interactions that are so vital for overall physiological function of the tissue. The reversibility of such changes is clearly of fundamental importance. However, once cells are committed down a pathway of cell death, there is perhaps little that can be done to reverse this process. The terminology applied to describe cell death perhaps clouds our perception and too easily leads us to categorize cell death simply as apoptosis or necrosis. In particular, the term necrosis merely describes an end point and sheds no light on the mechanism by which a cell died, for example by oncosis. Understanding the mechanisms and pathways to cell death may, however, provide insights into novel targets for supporting cells, reducing cell loss, and improving functional integrity.

• 2674

**Evaluation of the donor tissue during storage**

BORDERIE V

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Development of new surgical techniques for corneal transplantation induces major modifications in the graft selection process in eye banks. Quality of the donor corneal tissue must currently be assessed according to the scheduled surgical procedure. Not only the endothelial layer should be assessed by conventional techniques (trypan blue staining, light microscopy, specular microscopy) but also the corneal stroma and epithelium. This latter task requires development of new techniques such as high definition optical coherence tomography. Full field-OCT, in providing a detailed study of corneal structures with ultrahigh resolution (1 µm in all directions), permits detecting corneal conditions that are currently difficult to identify in eye banks, such as keratoconus, absence of Bowman's layer, or corneal scars. It permits evaluating precisely Descemet's membrane condition. It seems to be a promising technique in complement to current assessment methods of human donor corneas. Proper evaluation of the donor tissue by the new technologies implies formation of the eye bank staff. The staff has to get close relationships with the surgeons and to be attentive to the special requirements related to the scheduled surgical technique.

**Joint Meeting: EEBA symposium: storage and processing of donor tissue before transplantation**

• 2675

**Preparing donor tissue for DSEK**

DICKMAN M

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Donor tissue preparation remains a critical step in endothelial keratoplasty technique. Since its introduction in 2008 the use of pre-cut grafts for Descemet stripping automated endothelial keratoplasty (DSAEK) has become increasingly popular. Pre-cut tissue enables additional quality control, not readily available in the operating theater, shortens operation time, eliminates intraoperative donor related complications, overcomes surgeon's learning curve associated with valuable donor tissue loss and allows surgeons who do not have the necessary equipment to perform the procedure. Previous reports suggesting thinner grafts may result in better visual outcomes have driven surgeons to either try and harvest ultra-thin grafts themselves or request eye banks to do so for them. However, preparation of ultra-thin grafts is not without challenges. In the current session the literature on the safety and efficacy of pre-cut tissue for (ultra-thin) DSAEK will be reviewed. The results of our in-vitro studies on thickness reproducibility and endothelial cell viability of ultrathin pre-cut tissue for DSAEK, using an innovative microkeratome system, as well as our preliminary clinical experience with these grafts will be presented.

• 2676

**Descemet's membrane preparation in eye banks for DMEK**

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Eventhough Descemet Membrane Endothelial Keratoplasty (DMEK) results in unsurpassed improvement of postoperative visual acuity the complexity of graft preparation in DMEK prevents the wide spread use of this technique. Precut tissue preparation in eye banks for DMEK would make this technique amenable to a larger group of surgeons. Precut preparation of organ cultured corneas was preformed as previously described resulting in incompletely stripped Descemet's membranes where a small central part was still attached to the corneal stroma. After subsequent culture for up to 5 days the pre-cut grafts were used for DMEK. Visual acuity, central corneal thickness and endothelial cell density were retrospectively analyzed. Stripping was successfully completed in all grafts directly prior to surgery. The average visual acuity (logMAR) improved from 0.47 + 0.11 preoperatively to 0.2 + 0.13 3 months after DMEK. Central preoperative corneal thickness decreased from 632 + 46 µm preoperatively to 471 + 7 µm 3 months after DMEK. Average endothelial cell density was 2326 + 119 cells /mm<sup>2</sup> before and 2226 + 150 cells/mm<sup>2</sup> 3 days after pre-cut preparation (2 days before DMEK). 3 months after DMEK endothelial cell density further decreased to 1519 + 57 cells/mm<sup>2</sup>. Precut preparation of DMEK tissue, generated by incomplete Descemet stripping, leads to minor endothelial cell loss during subsequent culture and results in a fast visual recovery, only minor additional endothelial cell loss and a rapid decrease of corneal thickness.

• 2677

**Revisiting corneal storage using an innovative bioreactor**

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Be it at 4°C or in organ-culture at 31°C, two techniques no revisited since 70' corneal storage is mandatorily accompanied by a significant endothelial cell (EC) death (600x more than during lifetime and stromal swelling responsible for endothelial folds triggering EC apoptosis. The absence of intra ocular pressure that constitutes one of the main forces opposed to the naturally hydrophilic stroma may play an important role in this vicious circle that begins with donor death and is prolonged during storage. The restoration of pressure gradient with circulation of fluids during long term storage may improve EC survival and reduce stromal swelling. Review of the literature and of patents on methods available to achieve ex vivo restoration of the corneal physiology and personal works of our laboratory of bioengineering with a patented corneal bioreactor. Several devices have been published or patented previously but none with the aim of improving storage (generally for short-term toxicologic studies) and none using the technical solutions that we developed. Restoration of an increased pressure at the endothelial side associated with a continuous renewal of storage medium allowed rapidly reduction of stromal swelling and improvement of EC viability, compared to the rustic immersion in a sealed flask. Our innovative bioreactor could be used for research studies as well as for corneal storage in a next future in eye banks. Grant: Agence de la BioMédecine appel à projet recherche et greffe 2012

## SIS: Controversies in the pathogenesis and treatment of retinal vein occlusion

### • 2711

#### Controversies in the pathogenesis of RVO – Local perspective

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BRVO usually occurs at an arteriovenous (AV) crossing site, where a common adventitial sheath binds the artery and vein together. Several findings seem to demonstrate that vitreoretinal traction might have a significant role in some cases of BRVO. This prospective observational case-control study, with SD-OCT directed to the occlusion site, analyzed the prevalence of vitreoretinal traction in twenty BRVO patients. The results showed a true vitreoretinal traction in 7 eyes (35%). 5 eyes (25%) were associated with an adherence of posterior hyaloids without signs of retinal traction, whereas 8 eyes (40%) had neither vitreoretinal adherence nor vitreous traction. Regarding the same vessel segment of the fellow eye, none of the cases (0%) revealed vitreoretinal traction in the correspondent AV crossing site; 9 cases (45%) presented vitreoretinal adherence; and the remaining 11 cases (55%) showed neither traction nor adhesion. Chi-square analysis indicated that vitreovascular traction in the occlusion site was significantly associated with BRVO ( $p<0.05$ ). In conclusion, a common firm vitreoretinal adhesion at the obstruction site is reported herein, pointing out the role of vitreovascular traction in the etiology of some cases of BRVO.

### • 2712

#### Controversies in the pathogenesis of RVO – Systemic perspective

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Although retinal vein occlusion (RVO) has been known for about 150 years, and traditionally was assumed to be one entity, there are many arguments that RVO actually is not one disease. It has been suggested that it actually consists of six different clinical entities, each with different clinical characteristics, prognosis and management. For example, central retinal vein occlusion (CRVO) etiologically, clinically and from the management point of view is totally different from branch retinal vein occlusion (BRVO), and that is also true in the case of the major and macular retinal vein occlusions. Similarly, it is well-established now that CRVO is of two types, ischemic and non-ischemic, with very different clinical characteristics and management. It has also been shown that pre-existing ocular hypertension or glaucoma are important risk factors for CRVO and hemi-central retinal vein occlusion but not for BRVO. Many risk factors indicate systemic nature of some forms of the disease, although their real value remains speculative. Therefore, the lumping them together as one disease might be incorrect and misleading. Taking all above into account the traditional single disease model of RVO should be re-evaluated.

### • 2713

#### Anti-VEGF agents in the treatment of RVO

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Vascular endothelial growth factor (VEGF) is a key player in the development of macular edema (ME) in patients with retinal vein occlusion (RVO). However various other factors influence the development of ME. In a rat model, we observed a fast and transient upregulation of the expression of VEGF, which is dominant for the acute stage of ME. In chronic ME an upregulation of inflammatory factors is presented. The intravitreal drugs reduce the ME, but the mechanism of action differs between steroids and anti-VEGF. Anti-VEGF drugs inhibit the VEGF whereas steroids presents an unspecific anti-inflammatory effect inhibiting the expression of broad spectrum of molecules. The determination of the patients who benefit from each particular treatment would improve the functional results and reduce the possible side effects. In a retrospective study in which we investigated whether BRVO patients benefit more from injection of bevacizumab or triamcinolone if treated according to the duration of ME we found out a better visual gain under treatment with bevacizumab in the first 4 months. In patients with treatment later than 4 months since onset of BRVO no significant differences between bevacizumab and triamcinolone were observed.

### • 2714

#### Intravitreal steroids to treat the RVO-associated macular edema

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Intravitreal steroids are interesting tools to treat macular edema associated to RVO. The effect of the treatment lasts approximately 4-5 months. The main risk is increased ocular pressure ranging from 15% to 50% according to the type of steroids. Patients with glaucoma treated with at least two medications or unbalanced glaucoma will be treated with another therapeutic option to avoid complications. The regular follow-up of the patient will define efficacy and safety of the treatment. For re injections, the rhythm of control can be slightly eased.

**Commercial interest**

**• 2715****The role of autologous plasmin enzyme in the therapy of macular edema secondary to RVO**

UDAONDO P

**ABSTRACT NOT PROVIDED****• 2716****Controversies in the surgical treatment of RVO - Associated macular edema***GARCIA - ARLIMI J, VELAZQUEZ VILLORIA D  
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Macular edema is the main cause of decreased visual acuity in retinal vein occlusions (RVOs). Most approaches to the management of RVOs, such as grid macular laser photocoagulation and intraocular injections of drugs, are addressed to reduce the permeability of the macular vascular net and to increase the vitreoretinal fluid exchange of oxygen and protecting factors. However, we need to solve the primary mechanism of the disease: the vein occlusion. In order to reperfuse the thrombosed vein, Radial Optic Neurotomy (RON) is a surgical procedure aimed at solving the "compartment syndrome" that may exist in eyes with CRVO or hemicentral RVO. Sheathotomy specifically releases the compressive factor at the arteriovenous crossings by sectioning the adventitial sheath. Recombinant tissue plasminogen activator (r-tPA) has been administered by several routes, including systemic, intravitreal and by endovascular cannulation of retinal vessels. PPV with peeling of the internal limiting membrane has also been suggested. Surgery remains a good treatment option for patients with very recent and edematous occlusion with poor visual acuity, as reperfusion of the vein is the best approach to treat the macular edema and avoid ischemic complications.

• 2721

**24 hour continuous ocular tonography Triggerfish and biorhythms of the cardiovascular system functional parameters in healthy and glaucoma populations**

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**Purpose** Rating circadian biorhythms of the eye-wall tension and cardiovascular system functional parameters in normal subjects(N) and patients with primary open-angle glaucoma(POAG), normal tension glaucoma(NTG), ocular hypertension(OH).

**Methods** Study population: 50 Ns, 30 POAGs, 10 NTGs and 7 OHs. Simultaneous, 24 hours continuous ocular tonography Triggerfish and ECG/Blood pressure Holter recordings. The comparison in 4 time intervals, set basing on time of falling asleep(F) and awakening(W). Data are compared within intervals (IN) 1:(F-5hrs,F], 2:(F,F+3], 3:(F+3,W], 4:(W,W+5]

**Results** Similar eye-wall tension profiles for N and OH were detected, both decreasing in IN4, OH shows higher values in IN3. POAG and NTG tension profiles are different in IN4 with noticeable change for POAG with its value increase in opposite to others. Cardiovascular parameters show the similar characteristics of SAP for all cases with the highest value for OH and the lowest for N. The recordings for DAP show the noticeable difference for NTG, where the profile is changing rapidly between the highest values in INs2 and 4 to the lowest one in IN3. Additionally, only for OH the IN3 value is higher than IN4 one. The comparison of heart rate profile shows a noticeable difference for POAG. All data will be presented

**Conclusion** The study showed specific, group dependent, biorhythms for 24-hour circadian eye-wall tension and functional parameters of cardiovascular system. These figures bring new insights into the pathogenesis of glaucomatous neuropathy and indicate the location of potential handle points for individual time-dependent therapeutic options.

• 2722

**The therapeutic impact of office-hours diurnal pressure measurements in the management of glaucoma patients**

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**Purpose** To assess, primarily, the impact of office-hours diurnal IOP measurements on our clinical treatment attitude for glaucoma patients. Secondly, to determine, specific risk factors which allow us to select the group of patients where performing an IOP diurnal curve can be useful.

**Methods** 20 patients were included in this retrospective study. We examined the files of the patient who underwent a diurnal IO pressure curve between 01/01/2012 and 01/03/2013. Age, gender, race, diagnosis, the ophthalmological surgical and medical history, number of IOP measurements and the length of the diurnal curve on the examination day, the min and max IOP, and finally the therapeutic impact of these measurements and change in the treatment of patients.

**Results** The mean age was  $58.6 \pm 14.8$  years. There was min 4 to max 5 IOP measurements during  $8.4 \pm 0.5$  hours. The mean IOP of all the patients was  $17.7 \pm 3.2$  mmHg with a variation  $2.3 \pm 1.4$  mmHg (1 to 6 mmHg max) during the day. There were only 50% of the measurements which led to a change of the clinical treatment. In this group of patients the higher max IOP during the day and the higher IOP variation were the common feature.

**Conclusion** In our study we found that only 50% of the office-hours diurnal intraocular pressure curve led to a new clinical treatment. The patient selection should be stricter because of the time-consuming examination. Patients with higher IOP should be taken into account for diurnal IOP measurements where there are signs of progression.

• 2723

**Pseudoexfoliation: normative data and associations. The Central India Eye and Medical Study**

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**Purpose** To assess the prevalence of pseudoexfoliation (PEX) and its associations in a population-based setting.

**Methods** The population-based Central India Eye and Medical Study included 4711 individuals. All study participants underwent a detailed ophthalmological examination. After medical pupil dilation, PEX was assessed by an experienced ophthalmologist using slit-lamp based biomicroscopy.

**Results** PEX was detected in 87 eyes (prevalence: 0.95-0.10% (95%CI:0.75,1.15) of 69 subjects (prevalence: 1.49-0.18% (95%CI:1.14,1.83). In multivariate analysis, PEX prevalence was associated with higher age ( $P<0.001$ ; OR:1.11), lower body mass index ( $P=0.001$ ) and higher diastolic blood pressure ( $P=0.002$ ). In the multivariate analysis, PEX was not associated with retinal nerve fiber layer cross section area ( $P=0.76$ ) and presence of open-angle glaucoma ( $P=0.15$ ). Side differences in the presence of PEX were not significantly associated with side differences in intraocular pressure ( $P=0.40$ ).

**Conclusion** In a rural Central Indian population aged 30+ years, PEX prevalence (mean: 1.49-0.18%) was significantly associated with older age, lower body mass index and higher diastolic blood pressure. It was not significantly associated with optic nerve head measurements, refractive error, any ocular biometric parameter, nuclear cataract, early age-related macular degeneration and retinal vein occlusion, diabetes mellitus, smoking, and dyslipidemia.

• 2724

**Intraocular pressure and central corneal thickness in an old French population: The MONTRACHET study**

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**Purpose** To describe the distribution of intraocular pressure (IOP) and central corneal thickness (CCT) in an old French population.

**Methods** 9294 individuals 65 years and older were included in the 3C cohort study since 1999 in 3 French cities (Bordeaux, Dijon and Montpellier). In Dijon, an additional ophthalmic examination was performed 10 years after the initial inclusions to assess the relation between systemic age-related diseases and eye diseases in the MONTRACHET Study (Maculopathy Optic Nerve nutRition neuroAsCular and HEArT diseases). In this population-based study a thorough eye examination and a questionnaire were undertaken in each participant. Intraocular pressure (IOP) was measured with a noncontact pneumotonometer and central corneal thickness (CCT) was measured with an ultrasound pachymeter.

**Results** Among the 1153 participants of the MONTRACHET study only those without an ocular hypotensive treatment and those without evidence of glaucoma based on optic disc photography were considered. Among these individuals, 64% were female and the age was  $82.2 \pm 3.8$  years. IOP in the right eye and the left eye was  $14.6 \pm 3.2$  and  $14.8 \pm 3.2$  mmHg, respectively,  $p = 0.06$ . IOP was lower in pseudophakic than in phakic participants  $15.2 \pm 3.2$  and  $15.4 \pm 3.1$  mmHg vs  $13.9 \pm 3.0$  and  $14.1 \pm 3.1$  mmHg for the right and the left eye, respectively,  $p < 0.01$ . CCT in the right eye and the left eye was  $553.4 \pm 34.4 \mu\text{m}$  and  $555.7 \pm 34.7 \mu\text{m}$ , respectively,  $p < 0.01$ . CCT was not influenced by the lens status and the refraction error.

**Conclusion** These preliminary results of the MONTRACHET study shed a new light on the relations between IOP and the lens status in the elderly.

• 2725

**Dependence of the origin of pain in the eyes with terminal stage of glaucoma from chemical composition of vitreous**

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**Purpose** To identify a possible link between the origin of hypertonic pain syndrome (HPS) in the eyes with terminal stage of glaucoma and the difference of the urea concentrations between vitreous contents and blood serum.

**Methods** 31 patients (31 eyes) with refractory terminal glaucomas of different origins with IOP more 35 mm Hg were studied and operated on. The principle of surgery was drainage of the vitreous space for HPS elimination. Some vitreous contents and venous blood were taken during the surgery and researched for measuring urea concentrations. The samples were centrifuged then the blood serum and the supernatant of the vitreous contents were analyzed (SPOTCHEM-EZ SP 4430). The patients were divided into 3 groups depending on the intensity of pain syndrome: 1st with strong pain; 2nd - pain was present earlier; but it was absent before the surgery; 3d - pain was never registered.

**Results** A high concentration of urea in vitreous contents was found. Only in 6.45% the urea concentration in blood serum was higher than in vitreous. In 12.9% concentrations were near equal and in 80.64% the urea concentration in vitreous contents was higher than in blood serum. Pain existed in the cases when the difference (the negative gradient) of urea concentrations exceeded 6.1 mmol/L only.

**Conclusion** High concentration of the osmotically active urea in the vitreous cavity can be the reason for increase of IOP in some cases of refractory glaucomas. If the negative gradient of the urea concentrations were higher than the critical level, it would be the reason for the development of HPS.

• 2726 / T061

**Cognitive function associated with larger optic nerve heads**

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(1) *Ophthalmology, Mannheim*

(2) *Beijing Institute of Ophthalmology, Beijing*

**Purpose** To assess associations between optic nerve head size and parameters of cognitive function in a population-based study design.

**Methods** The Beijing Eye Study is a population-based cohort study in Northern China and included 4442 subjects. The study participants underwent a detailed ophthalmologic examination and an interview with questions on the level of education. Using fundus photographs, we measured the size of the optic disc.

**Results** Assessable optic disc photographs were available for 4089 (92.1%) subjects. After adjusting for age, gender, refractive error, and best corrected visual acuity, optic disc size was significantly associated with a higher level of education ( $P<0.001$ ) and shorter time needed to perform frequency doubling threshold perimetry ( $P<0.001$ ). In a reverse manner, the level of education was significantly associated with increasing optic disc size ( $P<0.001$ ) and shorter perimetric test duration ( $P<0.001$ ) after adjustment for age, gender and best corrected visual acuity.

**Conclusion** In a population-based study on adult Chinese, optic nerve head size was associated with the highest achieved level of education and time needed to perform a standardized perimetric test. Considering level of education and time needed to perform perimetry as surrogates of cognitive function, optic nerve head size was related to cognitive function.

• 2727 / T062

**Spectral analysis of ocular pulse amplitude recordings obtained using a contact lens sensor**

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(2) *NIHR Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London*

(3) *Optometry and Visual Science, City University London*

(4) *Ophthalmology, University Medical Centre, Mainz*

**Purpose** The aim of this study was to investigate whether the ocular pulse amplitude (OPA) can be detected in fluctuation curves recorded with the Sensimed Triggerfish® Sensor (TS), and analysed by Fast Fourier Transform (FFT).

**Methods** 40 subjects (20 open angle glaucoma [OAG], 20 healthy) underwent one hour of TS monitoring. Intraocular pressure and OPA were measured by dynamic contour tonometry (DCT) and DCT OPA was used as the reference. Segments of TS OPA fluctuations were visually identified (VI), and the amplitude (mVequ) was measured (VI-TS OPA). In addition, FFT signal analysis, which reduces a periodic function into its frequency components, was performed on VI segments, and the amplitude (arbitrary units [AU]) of the fundamental measured (FFT-TS OPA). The correlation between VI- and FFT-TS OPA was investigated. VI- and FFT-TS OPA were compared with DCT OPA.

**Results** In 3 subjects, TS recording failed. In 8 subjects, either no OPA or no fundamental could be identified. For the remaining 29 subjects (13 OAG, 16 healthy), both mean ( $\pm$ standard deviation) VI- and FFT-TS OPA were lower in OAG ( $9.0 \pm 3.9$  mVequ and  $184.6 \pm 303.7$  AU) than in healthy subjects ( $10.2 \pm 4.5$  mVequ and  $303.3 \pm 292.0$  AU). Mean DCT OPA was higher in OAG ( $2.7 \pm 0.8$  mmHg) than in healthy subjects ( $2.3 \pm 0.6$  mmHg) ( $p>0.05$  for all). There was a significant correlation between VI- and FFT-TS OPA (Spearman's rho=0.84;  $p<0.0001$ ). In OAG subjects only, the correlation between VI-TS OPA and DCT OPA (Spearman's rho=0.52,  $p=0.072$ ) and FFT-TS OPA and DCT OPA (Spearman's rho=0.48,  $p=0.099$ ) approached significance.

**Conclusion** It is possible to identify OPA in TS fluctuation curves and to analyse OPA by FFT. There is a weak association between TS OPA and DCT OPA in OAG.

## SIS: The quality of vision: the new frontier of modern ophthalmology

### • 2731

**Why is a precise assessment of the quality of vision essential in dry eye patients?**

PISELLA P  
Tours

**ABSTRACT NOT PROVIDED**

### • 2732

**3D or not 3D: The binocular tolerance to higher order aberrations**

GICQUEL JJ  
Poitiers

For the last decade, thanks to the measurement of higher order wavefront aberrations, we have been able to precise the concept of quality of vision (a new frontier that goes beyond visual acuity). These techniques, first developed for astronomical applications and defense, gave the ophthalmologist new tools, to measure the quality of vision, in healthy subjects and patients. We will go thru the pros and cons of each of the devices, developed for this very purpose and show their current applications. We will finally present new techniques, studying the quality of vision dynamically, in 3D and giving the patient a "preview" of a potential surgical procedure on his quality of vision.

### • 2733

**Should our decision to practice cataract surgery be only based on visual acuity?**

VAN DEN BERG TJTP  
Amsterdam

An obvious minimum criterion for cataract surgery is the likelihood that the surgery will be beneficial to visual function of the patient. Visual function is in practice often simplified to visual acuity VA. Although, most surgeons agree that VA is the most important parameter, it is also agreed there are other visual function factors to consider, corresponding to complaints such as glare, hazy vision, face recognition problems. This may suggest a complicated multidimensional array of visual functions to be important. To address this question in a comprehensive way, the functional point-spread-function is useful. The effect of cataract can be summarized by its small angle effect, dominating VA (and contrast sensitivity), and its large angle effect, corresponding to straylight SL. The question then is how important this large angle domain of visual function is for the cataract patient. Results of 2 studies will be presented, a questionnaire study in cataract patients, and a study of referral practice for CE. Subjective complaints were documented before and after surgery by the 39-item National Eye Institute Visual Function Questionnaire (NEI VFQ-39). Both studies show dominance of VA, but with an important second place for SL.

**Commercial interest**

### • 2734

**Spherical aberration: Friend or foe?**

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CHU Brettonneau, Tours

To determine the level of residual spherical aberration that gives the best objective and subjective quality of image after cataract surgery with intraocular lens (IOL) implantation. Six months after microincision (1.8 mm) cataract surgery with aspheric IOL implantation, total aberrations were computed using a Wavescan aberrometer. The modulation transfer function (MTF), Strehl ratio, and objective index of scattering were measured using the Objective Quality Analysis System. Objective depth of focus was computed as the focus range at which the Strehl ratio did not fall below 50% of maximum. Subjective depth of focus was calculated as the difference between the vergence of the punctum remotum and that of the punctum proximum. Thirty patients (54 eyes) were evaluated. The MTF cutoff values were higher with decreasing total ocular spherical aberration ( $r = 0.56$ ;  $P < .05$ ). Objective and subjective depth of focus were positively correlated with total spherical aberration ( $r = 0.26$  and  $r = 0.46$ , respectively;  $P < .05$ ). A final spherical aberration of zero obtained by compensation of IOL asphericity gave the greatest improvement in objective quality of vision and better MTF contrast. However, a final target ocular spherical aberration between 0.07  $\mu\text{m}$  and 0.10  $\mu\text{m}$  should be considered to be the best compromise between subjective depth of focus and objective contrast sensitivity.

**• 2735****The effectiveness of wavefront-guided refractive laser treatment**

SULEMAN H, FARES U, AL-AQABA MA, OTRI AM, SAID DG, DUA HS  
*Nottingham*

**PURPOSE:** To compare the efficacy, predictability, safety and induced higher-order aberrations (HOAs) between wavefront-guided and non-wavefront-guided ablations.

**METHODS:** The Cochrane Central Register of Controlled Trials, PubMED, and EMBASE were searched for randomized controlled trials. Trials meeting the selection criteria were quality appraised and data extracted by 2 independent authors. Measures of association were pooled quantitatively using metaanalytical methods. Comparison between wavefront-guided and non-wavefront-guided ablations was measured as pooled odds ratios (ORs) or weighted mean differences. The pooled ORs and 95% confidence intervals (CIs) were computed for efficacy, safety, and predictability. The weighted mean difference and 95% CIs were used to compare induced HOAs.

**RESULTS:** Eight trials involving 955 eyes were included. After wavefront-guided LASIK, the pooled OR of achieving uncorrected distance visual acuity (UDVA) of 20/20 (efficacy) was 1.10 (95% CI, 0.66-1.83;  $P=.72$ ), the pooled OR of achieving a result within  $\pm 0.50$  diopter of intended target (predictability) was 1.03 (95% CI, 0.60-1.75;  $P=.92$ ), and the weighted mean difference in induced HOAs was -0.09 (95% CI, -0.17 to -0.01;  $P=.04$ ). No study reported loss of 2 or more lines of Snellen acuity (safety) with either modality.

**CONCLUSIONS:** Metaanalysis showed no clear evidence of a benefit of wavefront-guided over non-wavefront-guided ablations. With high preexisting HOAs, however, wavefront-guided has advantages over non-wavefront-guided treatment.

## Course 11: Practical ophthalmic pathology - basics, updates and new insights

### • 2741

#### Tips and tricks in grossing & processing specimens

*COUPLANDS  
Liverpool*

Ophthalmic specimens range in size and degree of complexity. The diagnostic work-up of ocular-, ophthalmic biopsies and larger specimens demands close collaboration between the clinician, the pathologist and other specialties, including the vitreoretinal surgeon, plastic surgeon, as well as the head-and-neck surgeon. Documentation of all relevant clinical information in the pathology request form as well as timely discussions between the various specialists (e.g., telephone or E-mail communications prior to a biopsy being performed) are essential components of the diagnostic and referral pathways. The laboratory itself should be equipped with experienced technical staff who are dedicated to eye specimens and familiar with the specimen protocols. Further, a pathologist with expertise in ocular pathology/fluid samples should be supported with a wide range of investigations, including molecular diagnostic techniques. In this way, the yield from these samples can be optimized to reach an unequivocal diagnosis, rapid communication to the clinician, and timely instigation of therapy. Guidelines for these steps are provided.

### • 2742

#### Overview of conjunctival and eyelid tumours

*VAN GINDERDEUREN R (1, 2)  
(1) Ophthalmology, Leuven  
(2) Pathology, Leuven*

In this part of the course an overview will be given of the most common and most important conjunctiva and eyelid tumors ( basal cell carcinoma, squamous cell carcinoma, sebaceous cell carcinoma, malignant melanoma). For each disease clinical-pathologic correlations will be emphasized. Typical and atypical characteristics will be shown. In most cases the clinician finds the correct diagnosis, but even then it is important to investigate the section margins and the degree or details of the tumor. In rare cases a surprise in diagnosis is found and some important examples of these will be given, with also the useful consequences on the clinical follow-up of these patients. Correct diagnosis can also be useful in non-malignant processes and examples will be given. A short overview will be given of newer techniques and diagnostic possibilities.

### • 2743

#### Anterior to posterior “tour” of ocular disease processes

*VAN GINDERDEUREN R (1, 2)  
(1) Ophthalmology, Leuven  
(2) Pathology, Leuven*

In this part of the course the most important pathology of the internal eye structures will be explained from front to back side of the eye. The malignant tumors of adults (melanoma, metastasis , lymphoma) and children (retinoblastoma) will be covered, but also the differential diagnosis and important consequences of non-malignant diseases (sympathetic ophthalmia). Newer technologies can be incorporated in the pathologic examination which can have far reaching consequences for the patients. Enucleation and the difference with evisceration specimens will be discussed. Newer methods for fine needle aspiration biopsy (in solid tumors) and vitreal biopsies ( cells in the vitreal fluid) in malignant and inflammatory processes will be explained.

### • 2744

#### Overview of adult and paediatric orbital pathology

*HEEGAARDS  
Copenhagen*

An update of the most important orbital diseases both for adults and for children will be presented. Diagnostic hallmarks for the most important diseases will be shown and correlated to the clinical features.

**• 2745****Molecular techniques in ocular pathology***COUPLANDS S**Pathology, Dept. of Molecular and Clinical Cancer Medicine, Liverpool*

The roles of molecular techniques in ocular pathology include: a) aiding the understanding of disease pathogenesis; b) establishing diagnoses; c) predicting prognosis; d) predicting therapy response; and e) detecting residual disease. Examples of recent developments in molecular pathology include better understanding of the sonic hedgehog pathway in BCC; polyomavirus MCPyV in Merkel cell carcinoma; microsatellite instability in sebaceous carcinoma; fusion oncogenes in adenoid cystic carcinoma and mucoepidermoid carcinoma; and A20 gene deletions in conjunctival MALT lymphoma. Molecular techniques, such as IgH-PCR and TCR-PCR, are required regularly for the confirmation of the diagnosis of ocular B- and T-cell lymphomas, respectively. Molecular methods are used for prognostication in uveal melanoma, in combination with the clinical and histomorphological features of these tumours. The presence of monosomy 3 and polysomy 8 was initially detected using FISH but MLPA, aCGH, aSNP and GEP, have been introduced in various ocular oncology centres, providing more detailed information. It is hoped that next generation sequencing will provide potential targets for improved therapies in ocular malignancies.

**• 2746****Comparative ocular pathology and animal models used in eye research***HEEGAARD S**Copenhagen*

This lecture dealing with comparative eye pathology will present various interesting animal eyes seen from a basic anatomical point of view but also seen as a potential animal eye model used for research. Anatomical differences in different animal eyes will be presented and nature's way of solving different eye problems is discussed.

## SIS: Neuro-ophthalmic complications in the patient with cancer

• 2751

### Neuro-ophthalmic complications of cancer chemotherapy

KAWASAKIA  
*(1) Lausanne*

Cancer patients nowadays have longer periods of remission and higher rates of cure. The greater number of efficacious chemotherapeutics agents has no doubt contributed to prolonged survival rates for cancer patients but this has also led to a greater number of adverse and neurotoxic effects of chemotherapy. Sometimes, it is not easy to determine the exact etiology of a neuro-ophthalmologic disorder as these patients have multiple potential causes : metastasis, nutritional or metabolic derangement, vasculopathy, paraneoplastic syndrome, complication of radiation. Nevertheless, it is important for the ophthalmologist to have some familiarity with the more commonly reported neuro-ophthalmologic complications of chemotherapy. This lecture will discuss agents that have been associated with optic disc swelling, increased intracranial pressure, cranial nerve palsy and acute cerebellar syndrome.

• 2752

### Paraneoplastic syndromes

SZATMARY G (1), POLGAR TATJA (2)  
*(1) Hattiesburg  
(2) Neurology, Hattiesburg*

Neuro-ophthalmologic disorders in the patient with cancer  
Paraneoplastic syndromes  
Background: Paraneoplastic syndromes are caused by remote effect of cancer rather than direct invasion.  
Objective: Review of paraneoplastic neuro-ophthalmic manifestations, differential diagnosis, diagnostic workup and proposed pathophysiology will be presented.  
Methods: Literature review along with representative case presentations.  
Results: Overall incidence of remote effect of cancer is 10-15 % of all cancers. The number of autoantibodies in association with paraneoplastic syndromes is accumulating.  
Conclusion: Paraneoplastic syndrome with ocular manifestation may be the first sign of malignancy, and therefore ophthalmologist may play a crucial role in early detection and timely treatment with potential improvement of the disease course.

• 2753

### Meningeal carcinomatosis

SZATMARY G  
*Hattiesburg*

Neuro-ophthalmologic disorders in the patient with cancer  
Meningeal carcinomatosis  
Background: Meningeal carcinomatosis may be the first sign of malignancy, although usually represents widespread disease, and occurs most frequently in association with lung, breast and gastrointestinal cancer.  
Objective: To review the ophthalmological symptoms, signs, imaging appearance and differential diagnosis of meningeal carcinomatosis.  
Methods: Retrospective literature review and case reports.  
Results: Ocular manifestations are reported in 91% of patients with meningeal carcinomatosis. These include visual loss, diplopia, papilloedema, optic atrophy, and pupillary abnormality.  
Conclusion: Ophthalmologists and other eye care providers play a crucial role in the early detection of meningeal carcinomatosis.

• 2754

### Anterior pathway meningiomas

KAWASAKIA (1), BOSCHIA (2)  
*(1) Lausanne  
(2) Brussels*

Meningiomas can cause visual disturbance by interrupting the afferent or efferent pathway of vision. Optic nerve sheath and clinoid meningiomas cause optic neuropathy, cavernous sinus meningiomas cause ocular motor palsies and occipital meningiomas cause homonymous hemianopsias. Case examples , pearls for radiographic detection and issues related to management will be discussed

• 2755

### Radiation optic neuropathy

BORRUAT F

Hôpital Ophthalmique Jules-Gonin, Lausanne

Radiation optic neuropathy (RON) is a radionecrosis of the anterior visual pathway. It is a rare delayed and unpredictable complication of radiotherapy. RON presents with an acute painless and permanent visual loss. Both accurate and early diagnoses are mandatory as some specific therapies might benefit the patient. This presentation will summarize the physiopathology of RON with an emphasis on clinical diagnosis and therapeutic possibilities.

## Free papers MBGE 1/2: Genetics; Genotypes and Phenotypes

### • 2761

#### The eye screen of the German Mouse Clinic (GMC) – New genetic insights into eye development and ocular disorders

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(2) German Mouse Clinic, Helmholtz Zentrum München, Institute of Experimental Genetics, Neuherberg

(3) Chair of Experimental Genetics, Technische Universität München, Center for Life and Food Sciences, Freising-Weihenstephan

**Purpose** To summarize GMC - Eye Screen findings with about 350 tested mouse mutant lines.

**Methods** Eye morphology was analyzed by Slit Lamp Biomicroscopy/Scheimpflug Imaging and Ophthalmoscopy/Optical Coherence Tomography, eye size by Laser Interference Biometry, and the Virtual Optokinetic Drum was used for testing visual ability.

**Results** The GMC is a large-scale phenotyping center where mouse models for human diseases are analyzed in a standardized way. More than 550 parameters are investigated in 14 different areas including behavior, neurology, cardiovascular functionality and vision and eye development. In the past 12 years, we identified eye pathologies in 43 mutant lines of the GMC workflow. The majority of them exhibited irregular eye sizes (72%), followed by morphological changes as corneal and lens opacities (21%) or retinal damages (16%) and moderately reduced visual abilities (7%). Overall phenotype predictions (EuroPhenome database; <http://www.europhenome.org/>) indicated that 89% of these lines were additionally affected in behavioral and/or neurological parameters, pointing to general relations between eye and brain development. Moreover, 46% showed heart impairments, connecting eye diseases with cardiovascular risks. Our data further proved for 11 gene products a previously unknown participation in ocular processes. These include the chromatin modifiers Asxl1 and Mysm1, the transcription factor Nfyα as well as Arvcf, Jmjd5 and Slc20a2 with a role in cell-cell communication, protein hydroxylation and phosphate transport, respectively.

**Conclusion** The GMC - Eye Screen is an effective approach for improving knowledge about ocular genetics and detecting associations with non-ocular disorders.

### • 2763

#### Pilot study in Polish keratoconus patients using next generation sequencing

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(2) Department of Genetics and Pharmaceutical Microbiology, Faculty of Pharmacy, Poznań University of Medical Science, Poznań

(3) Department of Ophthalmology II, Medical Faculty, Medical University of Warsaw, Warsaw

**Purpose** Next generating sequencing (NGS) is a useful tool in disease etiology evaluation. Keratoconus is a multifactorial disease in which NGS technology may be applicable. Here we present a design of keratoconus study based on transcriptome analyzes in human corneas derived from keratoconus and control individuals from Poland.

**Methods** A total of 96 individuals from Polish population are included into this study: 48 keratoconus patients and 48 individuals without keratoconus phenotype. Illumina HiScan SQ system is applied in transcriptome analyzes in the study.

**Results** Total RNA from corneas for RNAseq and small RNA analyzes was extracted. Transcriptome analyzes were initiated.

**Conclusion** Since genetic factors involved in keratoconus etiology are in majority unrecognizable, the newest available technologies are necessary to be involved in further keratoconus research. Support: Polish Ministry of Science and Higher Education, Grant no. NN402591740 and National Science Centre in Poland, Grant no. 2012/05/E/N/Z5/02127.

### • 2762

#### High resolution retinal image analysis of unilateral retinitis pigmentosa using adaptive optics

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(2) Nippon Medical School, Tokyo

**Purpose** To report the findings of en face adaptive optics (AO) fundus imaging in eyes with unilateral retinitis pigmentosa(RP) patient.

**Methods** The both eyes from a unilateral RP patient, 52 y.o. female, was imaged using both an infrared AO camera (rtx1™, Imagine Eyes, France) and spectral-domain optical coherence tomography (SD-OCT – Cirrus®, Carl Zeiss Meditec, Germany). Full field scotopic and photopic electroretinograms(ERGs) were recorded according to the ISCEV standard. Normal funduscopic examination, Goldmann visual field and fluorescence angiography(FA) were also examined. The AO images acquired from the unilateral RP patient were analyzed on cone density and then compared to the AO data from the eyes of 21 healthy volunteers with a mean age of 36 years (range 20-57).

**Results** The unilateral RP patient showed bony spicule changes, vascular attenuation and decreased peripheral visual field in the right eye. FA showed granular hyperfluorescence in the post pole, mottling of RPE and atrophy of choroid capillary in the peripheral retina in the right eye. Her ERGs of the right eye showed non-recordable in all responses tested. OCT findings in the right eye showed preservation of the photoreceptor layer only in the foveal and parafoveal regions with loss in the more peripheral macula. All these results in her left eye were normal. AO data showed severe reduction of cone density in the right eye and the left eye showed normal cone density and regular cone mosaic.

**Conclusion** In unilateral RP patient, AO showed only one eye was affected even in the cellular level examination. The examinations using AO may be useful in diagnosis and better understanding of pathology and management of unilateral RP cases.

### • 2764

#### Role of O-GlcNAcylation of Sp1 in the pathogenesis of diabetic retinopathy

DONOVAN K, ALEKSEEV O, AZIZKHAN-CLIFFORD J  
Drexel University College of Medicine, Philadelphia

**Purpose** Central to the pathogenesis of diabetic retinopathy (DR) is vascular endothelial growth factor A (VEGF-A). Gene expression of VEGF-A is regulated by several transcription factors, including specificity protein 1 (Sp1). Sp1 is known to participate in angiogenesis and co-localizes with VEGF-A in the epiretinal membranes of DR patients. Sp1 is heavily O-GlcNAcylated by the enzyme O-GlcNAc transferase (OGT). This glycosylation modification affects its transcriptional activity, localization, stability, and protein interactions. Since O-GlcNAcylation of proteins is elevated under high glucose conditions, we investigated the relationship between O-GlcNAcylation of Sp1 and increased VEGF-A transcription.

**Methods** Hyperglycemia-exposed ARPE-19 (retinal pigment epithelial cells) and TR-iRBB (rat retinal microendothelial cells) were assayed for levels of VEGF-A by RT-qPCR and Western blot. VEGF-A protein and mRNA levels were measured in cells depleted of Sp1 or OGT by shRNA. Small molecule inhibitors of OGA and OGT were used to increase or decrease total O-GlcNAc. Chromatin immunoprecipitation was used to assess levels of Sp1 bound to the VEGF-A promoter.

**Results** Hyperglycemia caused increased VEGF-A transcript and protein expression in ARPE-19 and TR-iRBB cells. OGT or Sp1 depletion significantly abrogated these glucose-induced changes in both cell types, while minimally affecting basal expression. ChIP analysis showed that glucose increased the amount of promoter-bound Sp1.

**Conclusion** Glucose-induced increases in pan O-GlcNac in the RPE and vascular retina may be participating in the aberrant expression of VEGF-A in DR. The highly O-glycosylated Sp1 transcription factor may be responsible for early VEGF-A production in the retina.

• 2765

**Global microarray analysis and metabolic pathway profiling of a transgenic model of conditional, selective Müller cell ablation**

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(2) School of Mathematics and Statistics, Sydney

**Purpose** Müller cells, the principal glia cells in mammalian retina, play a critical role in retinal homeostasis. We have developed a transgenic Cre-Lox model for selective, conditional Müller cell ablation to examine the relationship between Müller cell dysfunction and retinal diseases. The purpose of this study was to examine differentially expressed genes and their related pathways with microarray analysis, then to profile metabolic pathways in areas of Müller cell ablation.

**Methods** Affymatrix microarray was performed on whole retina samples 1 week, 1 month and 3 months after induced Müller cell ablation. Data were analysed with limma package ( $p<0.05$ ) and qRT-PCR was used for array validation. Isolation of patches of Müller cell ablation was achieved by laser capture microdissection (LCM) and qRT-PCR was conducted on pathway related genes. Immunofluorescence microscopy was used to validate results.

**Results** Neuroprotection and apoptosis-related genes were upregulated 1 week after Müller cell ablation, angiogenesis, tight junction and metabolic-pathway related genes were downregulated later. Further analysis of glycolytic and mTOR pathways with tissue obtained by LCM revealed significant downregulation of genes related to these pathways in patches of Müller cell loss compared with controls. Immunofluorescent studies revealed that the downregulations mainly occurred in the photoreceptor segments, although Enolase1 colocalised with Müller cells.

**Conclusion** We found reduction of transcription and expression of proteins involved in key metabolic pathways in patches of Müller cell ablation. This study provides new insights into the relationship between Müller cell dysfunction and retinal diseases.

• 2766 / T086

**Genotype-phenotype correlation in two patients with posterior polymorphous corneal dystrophy 3**

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(3) University of Auckland, Auckland

**Purpose** To determine the molecular genetic cause of posterior polymorphous corneal dystrophy (PPCD) in four Czech probands.

**Methods** Extensive ophthalmological examination including Pentacam and specular microscopy, and direct sequencing of the ZEB1 coding region were performed.

**Results** Two novel frameshift mutations within ZEB1 were identified: c.2617dup in exon 8 in a 21-year-old female, considered to be most likely de novo in origin, and c.698dup in exon 6 in a 21-year-old male. The first case had mild changes consistent with a PPCD diagnosis and best corrected visual acuity (BCVA) bilaterally was 1.0. The corneal phenotype of the second case was more severe with a BCVA of 0.4 in the right and 0.05 in the left eye. Corneas of both probands were abnormally steep (keratometry readings  $K1 \geq 47.4$  D and  $K2 \geq 45 \geq 49.2$  D) with increased pachymetry values, but no pattern indicative of keratoconus. Specular microscopy in both patients revealed reduced endothelial cell density (range 1055- 655 per mm<sup>2</sup>). Both probands had a history of surgery for inguinal hernia; the male patient also reported the presence of hydrocele.

**Conclusion** Nucleotide changes within the coding region of ZEB1 underlie the pathogenesis of PPCD in 4 out of 23 (17.4%) Czech probands.

• 2767 / T085

**Analysis of mitochondrial sequences in patients with keratoconus**

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(3) Department of Ophthalmology II, Medical Faculty, Medical University of Warsaw, Warsaw

**Purpose** Keratoconus (KTCN) is thinning and anterior protrusion of the cornea. The etiology of KTCN remains unknown. Both genetic and environmental factors are associated with the disorder. The purpose of this study was to identify novel genetic factors by analyzing mitochondrial sequences in cases and controls from Polish population. There are available only a few analyses of some mitochondrial sequences in KTCN studies.

**Methods** A total of 96 individuals from Polish population were included into this study. Chosen mtDNA fragments of all individuals were sequenced.

**Results** Sequencing analysis of chosen mitochondrial genome fragments have revealed numerous alterations including several novel polymorphisms. No sequence variants segregated significantly more frequent with KTCN have been identified.

**Conclusion** Analysis of chosen fragments of mitochondrial genome in Polish patients have revealed numerous sequence variants, however our results do not support involvement of mtDNA changes in KTCN in Polish patients. The KTCN development does not depend on a single change in the gene, but on the accumulation of numerous sequence variants. The complexity of the genetic basis of KTCN causes the need to find another approach to further investigate the etiology of KTCN. Support: National Science Centre (Poland), Grant 2011/03/N/NZ5/01470

## SIS: Mechanisms of wound healing

### • 2771

#### Wound healing in health and disease

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Wound healing is a complex process involved in ocular surgery, trauma and pathogenesis of several eye diseases. Due to the delicate and sensitive structures of the eye wound healing is playing a essential role in ophthalmology. The transparency and optical properties of the cornea makes the mechanisms involved in the wound healing after the corneal surgery important understand and treat in detailed way. In glaucoma surgery well controlled wound healing process is as important for the creation of a functioning passage to aqueous humor out of the eye. The state of the ocular tissues is of great importance for the success of ocular surgeries and further points out the role of inflammation in wound healing processes. For example in case of glaucoma surgery the ultimate goal is to prevent ganglion cell death by lowering the intraocular pressure. Because ocular surfaces are the most important pathway for the topical glaucoma drugs and thus most vulnerable to the topical adverse reactions, they influence on the preoperative state of these tissues and thus the success of glaucoma surgery. The wound healing SIS is focusing in the mechanism involved in the wound healing in general and particularly in corneal and glaucoma surgery.

### • 2772

#### The role of wound healing in cornea

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(2) *Department of Ophthalmology, Tampere*

Corneal fibrosis contributes to the substantial health burden due to "corneal blindness", a leading cause of blindness world-wide. Molecular intra-cellular regulators of the cytoskeleton, suggests that this class of proteins may be targets for therapeutic development. In the transformation the usually quiet fibroblast-to-a myofibroblast morphology, the expression of  $\alpha$ -smooth muscle actin (SMA) is characteristic. As an excessive healing response, fibrosis is the result of the corneal keratocyte to myofibroblast transformation and may occur in response to trauma, infection as well as surgery. Using a mouse model of fibrosis following both infection with Pseudomonas and by trauma (an anterior keratectomy) it was shown using an array for the cytoskeletal regulators that one key molecule was highly unregulated. Moesin a member of the ERM Complex (ezrin/radixin/moesin) was highly unregulated. The use of siRNA applied by iontophoresis showed that the molecules characterizing fibrosis, such as alpha-SMA. The clinical development focuses on a method that can be applied topically to lessen or prevent fibrosis after corneal insult.

### • 2773

#### The mouse model of glaucoma filtration surgery: from understanding the wound healing response to applications as a tool for validating anti-fibrotics

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(4) *Glaucoma Service, Singapore National Eye Center, Singapore*

(5) *School of Materials Science and Engineering, Nanyang Technological University, Singapore*

Purpose: Failure after glaucoma filtration surgery (GFS) is attributed to fibrosis at the operated site. We have developed a mouse model for GFS so as to understand the wound healing process after this procedure and to test anti-fibrotics for future clinical application. Methods: Modified GFS was performed on mice and bleb survival was determined by slit lamp photography. Real-time PCR, antibody-based cytokine assays, flow cytometry, and immunofluorescent analyses were performed to determine gene and cellular changes. Results: The mouse model of GFS mimicked the clinical response to mitomycin C. Further analysis of the operated tissues revealed that the post-surgical wound healing response was separable into two distinguishable phases: an early "acute inflammatory" phase followed by a late "fibrotic" phase. Conclusions: Our data suggest that targeting the distinct phases of the wound healing process after GFS may be desirable in order to overcome the long term scarring response. Furthermore, the mechanisms of potential anti-fibrotics may now be understood in terms of their specific effects on each phase of the wound healing process. Paper presentation at Anatomy & Cell Biology, Special Interest Symposium.

### • 2774

#### Cell biological mechanisms of wound healing

*JÄRVINEN T*

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Purpose Tissue repair is among the most complex biological processes that occur during human life. After an injury, multiple biological pathways become activated and are coordinated through three distinct phases of inflammation, proliferation and maturation to yield the reparative response. What distinguishes different tissues in their response to insult is that some tissues, such as bone, can regenerate and replace the injured tissue with tissue that is identical to one that existed there before, whereas most tissues, including human skin, heal by a repair process where the original tissue is replaced by non-functioning tissue known as a scar. The purpose is to review the repair process hereMethods Skin wound healing can be considered as a model organism on which to base our understanding of cell and molecular biology of the tissue repair. Results Knowledge on the biology of wound healing might help us to unlock latent regenerative pathways and steer the reparative response towards regenerative. Conclusion The identification of regenerative pathways could potentially change our current medical practices on tissue injuries as much as the introduction of antibiotics did for infectious diseases in the twentieth century

*Commercial interest*

**Industry-sponsored symposium : Ocular surface and corneal damage: new outcomes****• 2841****Dry eye associated with ocular surface damages***SCHMETTERER L**Vienna***ABSTRACT NOT PROVIDED****• 2842****Confusing corneal ulcerations: Management of neurotrophic ulcers***MURAINEM**Rouen***ABSTRACT NOT PROVIDED****• 2843****New therapy for persistent epithelial defects - report of the first cases***UDZIELA M**Warsaw***ABSTRACT NOT PROVIDED**

**FRIDAY  
SEPTEMBER 20  
2013**

• 3211

**Choroidal thickness measurements and variations with age, gender in normal, myopia and high myopia**

COSCAS G (1, 2)

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To evaluate CT and variations along foveal horizontal scan and age related change in CT in normal and myopic Methods:Multi-center prospective study with 3 groups:30 Normal,24 Mild Myopia,31 High Myopia. Manual measurement followed choroid boundary selection(supra-choroid space,lamina fusca and outer limit of vessels. CT was measured at 500 µm intervals up to 1,500 µm temporal and nasal, manually by two graders independently. Statistical analysis evaluated CT at each location,the effects of age and myopia and grader agreement.Results: 85 eyes were qualified.Excellent grader agreement:intra-class correlation>0.97(Bland-Altman analysis).The mean CT was 248.2±78.5µm for Normal--age=58±18;247.0±85.4 for Myopia;age=45±20;131.5±70.9for High Myopia;age=54±13. The mean CT was significantly thicker for Normal and Myopia than High Myopia. CT was highly correlated across all test locations and slightly thicker underneath the fovea. The overall slope of age related mean change for the mean CT was -1.95 µm/year.The effect of age differed among the study groups. CONCLUSION: OCT measurements of CT are reproducible.Age and high myopia negatively affect CT.There was good agreement in CT measurement between RTVue and Spectralis.

• 3212

**Choroidal circulation measurements by laser Doppler fluxmetry**

POLIRNARAS CJ

Ophthalmology Group, Rothschild Memorial, Genève

Continuous real time Laser Doppler Flowmetry (LDF), has been used in humans, for the evaluation of the subfoveal choroidal circulation and the investigation of the regulatory processes of the choroidal blood flow in response to various physiological stimuli, as increases and decreases of the ocular perfusion pressure.A progressive decrease of the choroidal blood flow was observed with the aging and the progression of the age related macular degeneration. Abnormal regulation of the choroidal blood flow in patients suffering with neovascular AMD or Diabétic microangiopathy were also reported. The correlation of the subfoveal choroidal blood flow with the anatomical changes of the choroidal circulation as observed by OCT are under investigation.

• 3213

**Changes in choroidal thickness in adult onset foveomacular vitelliform dystrophy versus exudative AMD**

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Service Ophtalmologique Universitaire, Crétel

To compare choroidal thickness (CT) between eyes with adult onset foveo macular vitelliform dystrophy (AOFVD) eyes with age-related macular degeneration (AMD).5 group (38 eyes each) with AOFVD, fellow eyes with AOFVD wet AMD, dry AMD, age-matched normal subjects.Choroidal thickness was measured using enhanced-depth imaging optical coherence tomography (EDI-OCT) with Spectralis. Subfoveal choroidal thickness (SFCT) in each eye was analyzed with measurement of the vertical distance from the Bruch's membrane to the innermost choroid/sclera junction, at 500µm intervals up to 1500µm, from temporal to nasal and from superior to inferior to the center of the fovea.The difference between SFCT AOFVD (325.66 +/- 86 µm) versus CT in wet AMD (168.21 +/- 57.7 µm) and CT in dry AMD (158.77 +/- 68 µm) and versus elderly normal (238.33 +/- 83µm) are statistically significant, ( $p < 0.0001$ ). The difference found between the CT of the affected eye and the fellow eye in AOFVD (325.66 +/- 86 µm) is not statistically significant ( $p = 0.6$ ).This study demonstrates thickening of choroid in the eyes with AOFVD, in contrast with choroidal thinning observed in eyes with AMD. CT could become criteria for diagnosis and specific treatment AMD vs AOFVD

• 3214

**Evaluation of posterior limits of choroid**

UZZAN J

Rouen

Purpose: Recent studies have reported choroidal thickness measurements based on FD-OCT images. Lamina fusca (LF) is the natural anatomical outer boundary of the choroid. Unfortunately, LF is not always clearly visible in OCT images. The goal of this study is to assess the difference between LF boundary and posterior choroidal vessel (PCV) boundary.Methods: In a retrospective case review study, Horizontal B-Scan images, through the center of the fovea were reviewed and 96 eyes were included. The eyes were divided into 3 categories based on the characteristics of the choroid-sclera junction: SCS if suprachoroidal space is visible as a dark strand, LF if lamina fusca is visible as a bright strand, and PVC if only posterior vessel cavity boundary is visible. Results: For the 48 eyes in either SCS or LF, choroid thickness measurement differ by 26.9µm on average, ranging from 8 to 85 µm in individuals between the two boundaries. Conclusions: Choroid thickness measurement could be affected by boundary criteria. Posterior vessels boundary is the best method in 50% of cases but the presence of Supra Choroidal Space must be remembered. A common posterior boundary definition may be necessary to avoid significant bias in further studies.

**• 3215****Comparison of choroidal thickness between central serous choroidopathy and polypoidal choroidal vasculopathy**

PUCHE N, COSCAS F  
Paris

**PURPOSE:** To compare choroidal thicknesses among eyes with polypoidal choroidal vasculopathy (PCV), and central serous chorioretinopathy (CSC).  
**METHODS:** Patients with PCV and CSC underwent enhanced depth imaging optical coherence tomography (EDI OCT). A horizontal linear section comprising 100 averaged scans was obtained of each macula. The choroidal thickness was measured from the outer border of the retinal pigment epithelium to the inner scleral border. Seventeen healthy subjects (17 eyes) also underwent EDI OCT.  
**RESULTS:** A total of 17 eyes of 17 consecutive patients with CSC (17 male, mean age 50 years range 37-66 years) and 15 eyes of 15 patients with PCV were included in the analysis (8 male, mean age 69 years range 57-73 years). Mean choroidal thickness was  $437 \mu\text{m} \pm 97 \mu\text{m}$  in patients with CSC and mean choroidal thickness was  $335 \mu\text{m} \pm 53 \mu\text{m}$  in patients with PCV. The choroid was thicker in PCV and CSC patients than in controls (mean age 62 years range 55-72,  $219 \pm 35 \mu\text{m}$   $p<0.005$ ). However, there was significant difference in mean subfoveal choroidal thickness between CSC and PCV ( $p<0.005$ ).  
**CONCLUSION:** The choroid was thicker in eyes with PCV or CSC than in control

**• 3216****Variations of choroidal thickness in exudative AMD before and after treatment**

MAUGET-FAYSE M, COSCAS G  
Paris

**PURPOSE:** To evaluate choroidal morphological changes 3 and 6 months after intravitreal injection of Ranibizumab for exudative age related macular degeneration.  
**METHODS:** Retrospective, nonrandomized study, including patients with exudative AMD treated with Ranibizumab intravitreal injection and followed during 6 months. Non treated eye of AMD patients will be consider as control. All patients underwent a complete ophthalmological examination. At baseline, 3 months and 6 months, foveal and non-subfoveal areas were evaluated with Spectralis optical coherence tomography on both eye: under the fovea and at 500 and 1500  $\mu\text{m}$  nasally and temporally.  
**RESULTS** and **CONCLUSION:** All studied eyes had exudative AMD at baseline treated with at least 3 Ranibizumab (Three Ranibizumab followed by injections if necessary were done for each patients). The results will be presented during the meeting.

**• 3221****Using 'big data' to examine visual field follow up in glaucoma***CRABB D**Department of Optometry and Visual Science, London*

Monitoring glaucoma patients represents a significant burden on clinical services, with an estimated one million visits annually in the UK alone. Visual field (VF) tests are routinely used for detecting worsening of vision. We aimed to estimate VF loss during patients predicted lifetime by examining around 250,000 VF tests from three different glaucoma clinics in England. Patients were excluded if they had too few VFs or insufficient follow-up (less than 3 years). Levels of VF loss were summarised using the Mean Deviation (MD) index from each test. MD at diagnosis and MD loss during predicted remaining lifetime, using a linear rate of MD deterioration (dB/year) and residual life expectancy tables, were calculated and plotted on motion graphs. Most patients followed in clinics have stable disease. Four percent (95% confidence interval: 3 to 5%) of patients were predicted to be at risk of statutory blindness in their lifetime. Likelihood of a patient suffering serious visual impairment in their lifetime is linked to level of VF loss at presentation. These findings from retrospective analysis of 'big data' could help inform planning of follow-up in glaucoma and also illuminate the importance of detecting the disease in primary care.

**• 3222****Visual field testing in clinical practice - The role of age, stage and follow-up duration***JANSONIUS NM**University of Groningen, University Medical Center Groningen, Ophthalmology, Groningen*

Perimetry is the key diagnostic tool for monitoring glaucoma patients with an established visual field defect. As perimetry is demanding for both the patient and the organization, it is important to perform it as efficient as possible, that is, perform the right number of tests, schedule the tests at the right moments, and interpret the test results correctly. Practical guidelines will be given on optimal data acquisition and interpretation in clinical practice. The importance of an adequate monitoring depends on the risk of life-time blindness – and thus on age, glaucoma stage, and treatment. An easily applicable tool that can be used to estimate this risk will be presented.

**• 3223****Is there any place for non-standard automated perimetry in glaucoma monitoring?***CHAUHAN B**Dalhousie University, Halifax*

Several modes of non-standard automated perimetry have been introduced for the detection and the follow-up of glaucoma, with some techniques described over 30 years ago. The objective of these techniques was to allow clinicians to monitor glaucoma more effectively than standard automated perimetry. In spite of dozens of studies that report apparently favourable results with these techniques, their use has not become widespread, and arguably is decreasing. This presentation will review the evidence for the merit of non-standard automated perimetry and argue that given that the frequency of standard automated perimetry is well below practice standards, non-standard perimetric techniques have a limited role in glaucoma.

**• 3224****How imaging can be used for clinical decision-making?***GARWAY-HEATH D**London***ABSTRACT NOT PROVIDED**

**• 3225****Clinically sensible follow-up for glaucoma**

*LEMIJ H  
Rotterdam*

Detecting glaucoma progression is clinically challenging. It is unclear how structural and functional measurements are best used. Data from several long-term structure and function follow-up studies into glaucoma progression have revealed a poor overlap between the two, suggesting that one should ideally use both structural and functional measurements for detecting glaucomatous progression in individual cases, with the risk of overcalling and overtreating so-called progression. With ever more limited resources, however, clinicians are probably forced to restrict their measurements to as few exams as possible in individual patients, calling for sensible choices which measurements to perform. Structural measurements appear to be more useful in early, including preperimetric, glaucoma and functional measurements in more advanced glaucoma. Descriptions, drawings or 2D photography of the optic disc are, on the whole, probably of limited value, whereas standard automated perimetry and imaging of the retinal nerve fibre layer appear to be most useful. This will be discussed in an interactive, clinically oriented presentation. A limitation of this study is that scanning laser tomography of the optic disc has not been evaluated.

• 3231

### Conception and optimization of a corneal bioreactor

TRONE MC (1), CAMPOLMIN (1), GAUTHIER AS (1), NEFZAOUIC (1), BERNARD A (1), NAIGEON N (1), NANGOLIM-FOSSO T (1), PISELLI S (1), GARRAUD O (2), ACQUARTS (2), THURET G (1, 3), GAIN P (1)

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 (2) Eye Bank, French Blood Center, Saint-Etienne  
 (3) Institut Universitaire de France, Paris

**Purpose** Restoration of a trans-corneal pressure (P) gradient and continuous circulation of fluids on both sides of the cornea could improve endothelial cell survival and reduce stromal swelling and Descemet folding during long-term corneal storage. Aim: to present the technical requirements and the technical choices we made during the development of an innovative corneal bioreactor (BR)

**Methods** Main functions were defined with a process of functional analysis of needs in collaboration with eye bankers. Successive prototypes were built using 3D printing with biocompatible resins, and machining of PMMA blocks, and windows made out of optical grade glass were glued. Different types of connecting tubes were assessed. Efficiency was analysed in term of capacity of maintaining the cornea perfectly tighten in order to create 2 hermetic endo and epi compartments. P (adjustable from 0 to 50 mmHg with a mini-LCD screen for live display) was regulated by a continuous irrigation from a disposable infusion system associated with a P sensor and a micro-solenoid valve driven by a customized microcontroller

**Results** The 3 most challenging points were: 1/waterproofness between epi and endo chambers that require to use corneas with at least a 16 mm diameter regular scleral rim 2/biocompatibility of glues 3/ oxidation and alcalinization of storage medium caused by materials permeable to atmospheric gases. Since 2 years, 9 successive prototypes allowed selection of efficient solutions

**Conclusion** Our innovative BR will be useful for both laboratory research and corneal storage by eye banks in a next future. Grant: ABM 2012, EFS 2012, ANSM 2012.PG, GT and SA patented the BR

• 3232

### Setting up organ-cultured corneas pre-cutting by a French blood center-eye bank

CAMPOLMIN (1, 2), GAUTHIER AS (1, 3), MONTARD R (3), ACQUARTS (4), THEILLIERE C (4), PEIRERA S (4), GARRAUD O (4), HEZ (1), PISELLI S (1), THURET G (1, 2, 5), DELBOSC B (3), GAIN P (1, 2)

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 (5) Institut Universitaire de France, Bd St Michel, Paris

**Purpose** In France, the cutting of endothelial graft for DSAEK is almost exclusively performed by surgeons directly in the operating room whereas in the US more than 2/3 are now precut by eye banks (EB) technicians using 4°C storage. Among the French Blood Center EB, the EB of St-Etienne received the first agreement from the French National Agency for Medicines and Health products for precutting and distribution of corneas. Aim: to report our first experience with DSAEK precut by technicians using organ-cultured (OC) corneas

**Methods** OC corneas with an initial endothelial cell density (ECD) >2200 cells/mm<sup>2</sup> (by image analysis) were precut by two trained technicians using a Moria microkeratome. Corneas were stored in CMax (Eurobio) and deswelled in CJet (5% Dextran T500) 20h prior to pre-cutting. Central corneal thickness (CCT) was measured by ultrasound before and after cut with a single pass of the 350µm head. Microbiological testing was done with blood culture bottles 24h after precutting

**Results** Twelve corneas with initial ECD of 2724±269 cells/mm<sup>2</sup> (mean±SD), 19±4 days of OC and CCT of 548±50 µm were cut and sent to 4 centers (Feb to May 2013). Final CCT was 194±33µm (166 to 277). All surgeries were uneventful but rebubbling was necessary in 6 cases and 1 graft with eccentric trephination by the surgeon did never adhere

**Conclusion** Precutting of OC corneas by EB allows quality and microbiology controls. Three improvements are ongoing: OCT thickness map before, during and after cut, use of the microkeratome double-pass technique, and ink marking of the centre to guide final trephination by surgeons. Grants: ONDAINE, AOL CHU 2012

• 3233

### Optical measurement of dioptric power and transparency of cornea stored in bioreactor

PATAIA G (1), GALITHIER AS (1, 2), TRONE MC (1, 3), CAMPOLMIN (1, 3), BERNARD A (1), NAIGEON N (1, 4), NANGOLIM-FOSSO T (1), PISELLI S (1), ACQUARTS (5, 1), LEPINE T (6, 7), DELBOSC B (2), THURET G (1, 3, 8), GAIN P (1, 3)

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 (7) Laboratoire Hubert Curien (UMR CNRS 5516), Saint-Etienne  
 (8) Institut Universitaire de France, Bd St Michel, Paris

**Purpose** Quantification of dioptric power (DP) and transparency (T) of stored corneas remain almost unmet in daily eye bank practice. Complementary to T, DP could help screening donors having had refractive surgery and selecting the best donor/recipient refractive matching. During the development of an innovative corneal bioreactor (BR) (patented) for preclinical research and long-term storage, we took into account the possibility to integrate devices to perform these new quality controls. Aim: to present a device, named "frontofoco-graft-meter" (FGM) able to quantify DP and T of corneas in BR

**Methods** measure of DP was based on the principle of magnification measurement and for T on the transmitted light over incoming light ratio. The FGM comprised a light source + specific chart made of parallel black lines + digital camera. Human corneas in the BR were pressurised at 15mmHg, with medium circulation in endothelial chamber, whereas epithelial chamber was filled with air during measurements. DP was compared to the power map provided by OCT (Tomey, Casia), and T measures to results provided by our previous device called 'transparometer' (based on the analysis of modulation transfer function). Measurements were done by 3 operators in a masked fashion

**Results** For DP, agreement with OCT was good (within ±1 Dioptry). For T, measures were comparable with both devices. Inter-rater agreement was good for DP and T. FGM took a limited place and could easily be integrated in eye banks

**Conclusion** the new FGM provides objective and reproducible QC of DP and T of corneas placed in the BR. Grants: ABM 2012, ANSM 2012, EFS 2012

• 3234

### Endothelial and stromal quality control of corneas stored in an innovative bioreactor

GAUTHIER A (1, 2), TRONE MC (1, 3), CAMPOLMIN (1, 3), NEFZAOUIC (1, 3), BERNARD A (1), NAIGEON N (1, 4), NANGOLIM-FOSSO T (1), PISELLI S (1), DELBOSC B (2), ACQUARTS (5, 1), THURET G (1, 3, 6), GAIN P (1, 3)

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 (6) Institut Universitaire de France, Bd St Michel, Paris

**Purpose** We recently patented a bioreactor (BR) for corneal storage, based on the restoration of a transcorneal pressure gradient and a continuous fluid circulation. Being designed for experimental works and eye banking it comprises 2 windows enabling a right through observation of the cornea. Aim: to assess on human corneas placed in the BR, the possibilities of non-invasive quality controls (QC) of endothelial cells (EC) and of the stroma (thickness, dioptric power, transparency, detection of scars)

**Methods** EC QC were: 1/transmitted light microscopy (TLM) with a x10 very LWD objective after trypan blue + osmotic preparation directly performed in the BR; 2/observation with a macroscope (same preparation); 3/specular microscopy (SM, Konan and Hai) without preparation. Stromal QC were: 1/slit lamp; 2/OCT (Heidelberg and Tomey Casia); 3/customized transparometer (analysis of modulation transfer function), and 4/macroscope. Both quality of results and ergonomic aspects were considered

**Results** EC were visible with TLM and microscope at highest magnification but not with SM. Casia OCT allowed easy thickness mapping with a good agreement with US pachymetry performed as a control, and dioptric power measurements. Transparency could be controlled with the slit lamp and with our transparometer. The microscope visualized stromal scars

**Conclusion** EC and new stromal QC are now possible for corneas stored in the innovative BR. Other new complementary technologies of optical control are currently in development in our lab. Acknowledgments: EFS of Besançon Grant: ABM 2012, EFS 2012, ANSM 2012.PG, GT and SA patented the BR

**SIS: Tumours and pseudo-tumours of the pigment epithelium**
**• 3241**
**Congenital hypertrophy of the retinal pigment epithelium (RPE)**

ZOGRAFOS L  
Lausanne

Congenital hypertrophy of the Retinal Pigment Epithelium (CHRPE) is a tumour which may be confused with pigmented tumours of the choroid such as nevi or melanomas. These lesions are round or oval shaped, with borders that are well defined or scalloped. They present areas of atrophy which may increase in size. This increase in size of CHRPE is well documented and innocent. However, transformation in adenomas or even adenocarcinomas is also possible. In consequence, a periodical observation for Congenital hypertrophy of the Retinal Pigment Epithelium is generally recommended.

**• 3242**
**Isolated and combined hamartomas of the RPE**

SCHALENBOURG A  
Lausanne

RPE hamartomas are rare, presumed congenital proliferations of the RPE (isolated) or both the RPE and neuroretina (combined), without malignant potential. Isolated hamartomas are well delineated, small, pigmented and asymptomatic lesions, most often in the macular region and not requiring any treatment. Combined hamartomas are unilateral malformations consisting of a densification of the RPE and vascularized gliosis of the overlying neuroretina. Its location at the posterior pole is correlated with varying degrees of visual impairment, for which epiretinal membrane peeling remains controversial. Treatment is occasionally required in case of complications such as neovascular membrane, vitreous haemorrhage, retinal detachment or macular hole. Being benign, it is crucial that RPE hamartomas are differentiated from other pigmented lesions, and most importantly choroidal melanoma, to avoid unnecessary radiotherapy or even enucleation in the –usually young- patients that present them.

**• 3243**
**Congenital hypertrophy of the RPE related to familial adenomatous polyposis**

DESJARDINS L  
Paris

**Introduction:** Familial adenomatous polyposis is a rare condition found in 1/10000 births. Multiple bowel polyps can lead to bowel cancer if untreated. Multiple pigmented retinal lesions are present in 60 to 80% of patients with this condition. **Clinical presentation and imaging:** Fundus lesions are classified in five groups: ovals pigmented with halo, small pigmented round, large pigmented round, depigmented round and small hypopigmented. They can be grouped with a bear track aspect. The lesions are asymptomatic. A total number of at least four lesions is considered a good marker of the disease. **Angiographic aspect:** is only mask or window depending on the degree of pigmentation. **Pathology:** Histologically the lesions are made of one or several layers of pigmented hypertrophic epithelial cells. **Genetic:** familial adenomatous polyposis is linked to a germline mutation of the APC gene which is localized on the long arm of chromosome 5.

**• 3244**
**Irido-ciliary tumours of the pigmented and non-pigmented ciliary epithelium**

DAMATO B  
Ocular Oncology Service, Liverpool

Tumours of the pigmented and non-pigmented ciliary epithelium include adenoma and adenocarcinoma, which can be pigmented or amelanotic according to whether they arise in the pigmented or the non-pigmented ciliary epithelium. These tumours can invade the anterior chamber and corneal endothelium. They can be multinodular. Complications include pigment scatter, subluxation of the lens, cataract, vitreous seeding and haemorrhage. Necrosis can cause uveitis. Histological differentiation between adenoma and adenocarcinoma can be difficult. Malignant tumours can spread extraocularly to cause an epibulbar tumour or proptosis. Some tumours arise in blind, traumatized eyes. The differential diagnosis includes melanoma, melanocytoma, Fuchs' adenoma, epibulbar squamous cell carcinoma and medulloepithelioma. Diagnosis is best achieved by excision biopsy, which also provides a cure. Radiotherapy can be followed by persistent uveitis and glaucoma ('toxic tumour syndrome'). Treatment, if indicated, usually consists of excision, if possible conserving the eye with useful vision.

**• 3245****Controversies in the surgical treatment of combined hamartomas of the retina and retinal pigment epithelium**

GARCIA-ARLUMI J, VELAZQUEZ VILLORIA D  
Barcelona

Combined hamartomas of the retina and retinal pigment epithelium are rare benign tumors that may cause significant visual loss. Combined hamartomas are usually solitary, unilateral lesions located at the optic disc or posterior pole. They typically appear slightly elevated and have varying amounts of pigmentation, vascular tortuosity and epiretinal membrane formation. They are usually diagnosed in children or young adults with painless visual loss. Visual function varies with the location of the lesion. Direct involvement of the optic nerve, papillomacular bundle, or fovea may reduce visual acuity. If these structures are not directly involved, visual loss may result from macular distortion by retinal striae and epiretinal membrane. According to the Macula Society's report of 60 patients with combined hamartomas, the tumor was located on the optic disc and adjacent retina in 18% of patients, adjacent to the disc in 28% of patients, extending from the disc to the fovea in 10% of patients, in the macula in 38% and in the mid-periphery in 5% of patients. Macular location of the tumor is a predictor of poor visual acuity, found in 69% of macular tumors and only 25% of extramacular tumors. Histopathologically, combined hamartomas show evidence of hamartomatous malformation involving hyperplasia of RPE, glial cells, and blood vessels. Epiretinal membranes are an important cause of progressive visual loss, especially in eyes with macular tumors. Indication for epiretinal membrane removal in these patients is not well established, and cases reported in literature show different visual results. McDonald et al<sup>2</sup> indicated that vitrectomy was not beneficial in their 2 cases, whereas Mason<sup>3</sup>, Kleiner<sup>3</sup> and Stallman<sup>4</sup> found benefit in their cases. The surface glial membrane causing the retinal folding is an integral part of the tumor in some cases and accounts for the fact that surgical stripping of the membrane is difficult and has little chance of restoring central vision in such cases<sup>5</sup>. Surgical intervention should be selected carefully; full-thickness macular holes have occurred when the membrane is intrinsically woven into the lesion<sup>6</sup>. It has been speculated that OCT could provide useful information regarding potential visual benefit as the more intact retina on OCT might have better visual outcome than the completely disorganized, thickened retina<sup>7</sup>. Vitreous traction if present may also be detected, and thus help in selecting cases that may have a potential for visual improvement with vitrectomy and membrane peel to relieve traction.

**• 3246****Debate: Challenging cases of retinal pigment epithelium lesions: How to diagnose? How to manage?**

GARCIA-ARLUMI J, DAMATO B, DESJARDINS L, SCHALENBOURG A,  
ZOGRAFOS L  
Barcelona

**ABSTRACT NOT PROVIDED**

## Course 12: Cell biological techniques for eye research

### • 3251

#### The role of in vitro studies in eye research

*UUSITALO H (1, 2)*

(1) *SILK, Department of Ophthalmology, University of Tampere, Tampere*

(2) *Taub Eye Center, Tampere University Hospital, Tampere*

In vitro techniques are commonly used in eye research and they are valuable tools with several benefits like their modest price, well controlled test environment and the relatively simple interpretation of the results. They are after their validation usually relatively easily and quickly performed and in most cases suitable for automated analyses. In many cases their use can replace in vivo studies with experimental animals and thus they are ethically sustainable. By using valid in vitro techniques it is also possible to simplify the research frame from very complex in vitro situation and to set more specified hypothesis. There are, however, several drawbacks in the use of in vitro techniques. The results of them should be analyzed critically and the validity of the used in vitro technique should be rechecked on the basis of the new knowledge. It should also be kept in mind that an in vitro technique is usually valid for one specific cell mechanism and could be unacceptable for another.

### • 3252

#### How to study heterophagy in RPE cells

*SINHA D*

*Baltimore*

RPE is one of the most active phagocytic cell types in the body, phagocytosing 10% of total photoreceptor volume daily. Phagocytosis of the shed discs is essential to the survival of photoreceptor cells. This phagocytosis process can be divided into four distinct stages: 1) recognition and attachment of the OS; 2) ingestion of the OS; 3) formation of the phagosome and fusion with lysosomes; and 4) finally, digestion. Once OS are internalized by the RPE cells, the phagosome proceeds through a maturation process, followed by fusion with a lysosome to form a phagolysosome where degradation occurs. This pathway remains poorly understood in RPE. We have provided evidence that bA3/A1-crystallin has no apparent role in recognition or ingestion of the OS. However, loss of bA3/A1-crystallin in RPE has an impaired ability to degrade OS, perhaps resulting from a defect in the phagolysosomal pathway. The overall experimental design will be to determine the following parameters by modulating the expression of bA3/A1-crystallin in RPE: (1) Expression levels of proteins involved in phagosome maturation; (2) Interaction of phagocytic vacuoles with early and late endosomes; and (3) Phagosome-lysosome fusion as assessed by live cell imaging

### • 3253

#### Techniques to demonstrate immunoproteasome in retinal functions

*FERRINGTON D*

*Ophthalmology and Visual Neurosciences, University of Minnesota, Minneapolis*

The catalytic core of the proteasome contains three pairs of catalytic subunits that perform distinct proteolytic activities referred to as caspase-like, trypsin-like, and chymotrypsin-like. In the standard proteasome, the catalytic subunits are  $\beta 1$ ,  $\beta 2$ , and  $\beta 5$ . These subunits can be replaced in nascent proteasomes by the subunits LMP2 ( $\beta 1i$ ), MECL-1 ( $\beta 2i$ ), and LMP7 ( $\beta 5i$ ), which form the core of the immunoproteasome. The immunoproteasome is a proteasome subtype that is abundant in immune cells and also present, albeit in low concentrations, in cells outside the immune system. A well-described function of immunoproteasome is to generate peptides for MHC class I for display on the cell surface. Functions of immunoproteasome that go beyond its role in generating antigenic peptides are emerging from studies in immunoproteasome-deficient mice and from human diseases linked to mutations in immunoproteasome subunits. Notably, oxidative stress and pro-inflammatory cytokines, conditions that predominate in retinal diseases, are associated with immunoproteasome upregulation. Multiple methods used to investigate immunoproteasome function in the retina and in cultured retinal pigment epithelial cells will be discussed in detail.

### • 3254

#### Techniques for studying mechanisms of autophagy in RPE cells

*KAARNIRANTA K*

*Kuopio*

Autophagy is basic catabolic mechanism which "self eats" cellular components that are unnecessary or dysfunctional to the cell. Autophagy comprises three intracellular pathways in eukaryotic cells, which are macroautophagy, microautophagy and chaperone-mediated autophagy. These forms of autophagy are mechanistically different from each other, but finally they all lead to lysosomal degradation of intracellular material. Autophagy is triggered as an adaptive response during AMD-associated stress conditions such as hypoxia, oxidative stress, the unfolded protein response or inflammation. Macroautophagy process begins with the formation of isolation membranes called phagophores at the phagophore assembly site. The phagophores then become elongated and surround a portion of the cytosol to form mature double membrane autophagosomes that can engulf portions of cytoplasm containing oligomeric protein complexes and organelles. The autophagosomes fuse with the lysosomes and their content is then degraded by lysosomal enzymes. Failure of autophagy in aged cells may accelerates degenerative processes. Recent methods to study autophagy are discussed with details in the course.

## • 3271

**Conjunctival provocation test in ophthalmology daily practice***FAUQUERT J**Clermont-Ferrand*

Conjunctival provocation Test (CPT) is the main technique that may lead to the etiologic diagnosis of allergic conjunctivitis (AC). In most cases of seasonal and acute AC, the relationship with an allergen exposure is easily assessed. But in cases of perennial AC or in vernal as well as in atopic keratoconjunctivitis, the pertinence of the sensitization should be evidenced. To do this, CPT is the one tool available. The ophthalmologist plays a major role in its daily practice. Before CPT, a slit lamp examination is mandatory to avoid any contra-indication (elevated clinical scoring, corneal impairment). The rating of symptoms occurring during CPT requires the ophthalmologist's collaboration as well as management of reactions consecutive to the instillation of increasing doses of allergens. Thus CPT requires a narrow collaboration between the ophthalmologist and the allergist.

## • 3272

**Allergy and vernal keratoconjunctivitis: Results of a 251 children cohort***CHIAMARETTA F**Clermont Ferrand*

The aim of our study was to follow the evolution of phenotype for children with lattice corneal dystrophy type I and granular corneal dystrophy type I. Mean age was  $8,84 \pm 4,20$  years and  $17,78 \pm 3,76$  years respectively. Corneal parameters were analysed from Scheimpflug photographs: number of opacities, location, thickness, depth and percentage of corneal transparency. Peripheral opacities in LCDI children continued to increase when the number of central deposits tended to stagnate after a mean age of  $12 \pm 1$  years. GCDI children presented a continuous increase in number of opacities. Our results show a phenotypic heterogeneity, especially in early ages, which can lead to misdiagnosis.

## • 3273

**What's new in atopic keratoconjunctivitis***BREMONT-GIGNAC D**Amiens*

Specific clinical features of atopic keratoconjunctivitis are well known in adults and lead to a severe ocular surface impairment. However in children's keratoconjunctivitis, even if common vernal keratoconjunctivitis form predominates, some pediatric presentation differs and must be individualised. A complete clinical evaluation has to be precised for a better knowledge of the prognosis of the disease. Different new explorations physiopathological mechanisms are detailed in the pathology. This evaluation allows a better adjustment of the treatment.

**• 3321****Why is the optic nerve the canary in the coal mine of mitochondrial diseases?***SADUN A (1, 2)*(1) *Ophthalmology and Neurosurgery at USC Sch. Med., Los Angeles*(2) *Doheny Eye Institute, Los Angeles*

Mitochondrial Optic Neuropathies (MON) present characteristic clinical and pathological features. Loss of visual acuity, dyschromatopsia and a central scotoma speak to involvement of the papillomacular bundle (PMB). Histopathology confirms this. These features are seen in hereditary diseases (Leber's Hereditary Optic Neuropathy—LHON and Dominant Optic Atrophy), Syndromes (such as MELAS, Wolfram's or FA), or toxic MONs. So why is the optic nerve involved so early and so often in mitochondrial disease? The brain weighs 2% of the body but consumes 20% of the body's oxygen. This expensive consumption of energy is largely due to the need for repolarization of the axon's membrane after each action potential. This is largely mitigated by myelin such that the sodium/potassium pumps only work at the Nodes of Ranvier. However, the retinal nerve fiber layer remains unmyelinated due to the need for inner retinal transparency. We present a mathematical model of these conditions that predicts the order of fiber loss in MON as a function of myelin and axon diameter. We demonstrate, with morphometry of postmortem samples of normal and LHON optic nerves, that this pattern is strictly observed.

## • 3411

**RVO: A complex disease**

*PAQUES M  
Paris*

The events following a venous obstruction are poorly known, at the fundamental as well as at the clinical level. Experimental studies are poorly contributive, due to the questionable pertinence relative to the clinical situation. Despite numerous epidemiological studies, there is no strong consensus on the risk factors of RVO, especially the role of coagulation abnormalities, which appear to be modest at best. High blood pressure and glaucoma appears to be involved, however their pathophysiological role remains obscure. Controversies regarding the role of diabetes may be due to the fact that diabetics do more severe forms of RVO, hence a recruitment bias. The natural history of RVO is highly unpredictable at an individual level. Such poor predictability may arise from the intertwining of several parameters such as degree and duration of flow obstruction, blood pressure, intensity of microvascular remodelling... The aim of this SIS is to address some still unanswered questions about RVOs, to point controversies, and to propose new pathways for a better understanding and clinical management of these patients.

## • 3412

**Is there a need for a general workup of RVO patients?**

*HERON E  
Paris*

The pathophysiology of retinal vein occlusion (RVO) remains unclear, the precise cause of venous flow obstruction being still unknown. Established and potential risk factors of RVO, according to the published literature, will be reviewed and discussed with a rapid focus on thrombophilia. Recent new insight in the field of blood viscosity in RVO patients and its possible therapeutic implications will be presented. This will lead to conclude on which reasonable general work-up to be proposed to RVO patients.

## • 3413

**The issue of chronic macular edema**

*GIRMENS JF  
CHNO des Quinze-Vingts, Paris*

With availability of dexamethasone implant and ranibizumab, specifically indicated for the treatment of macular edema complicating RVO, a trend could be to treat as quickly as possible all retinal thickenings by intravitreal injections (more or less often, depending on duration of action of selected product). However, all retinal thickenings are not identical, nor from the same origin. Fine semiological analysis allows to distinguish different types : in addition to macular edema by blood-retinal barrier rupture (most common), we will describe other involved mechanisms, like macro-aneurysms, ischemia, diffusion of papilledema, epi-retinal membrane. Differentiation of mechanism is important because involving different therapeutic approaches.

## • 3414

**Toward a new classification of RVOs?**

*PAQUES M  
Paris*

The proteiform features of RVO challenges classification. The discovery of patients with abrupt visual loss due to severe hypoperfusion, manifesting by perivenular whitening (PVW) in the absence of nonperfusion, raises the question of the pertinence of the ischemic versus nonischemic classification. High resolution OCT showed that focal ischemia in these cases was restricted to the inner nuclear layer, the atrophy of which accounted for clinical sequelae. On the opposite, there are evidence that most cases of chronic macular edema are preceded by weeks or months of progressive venous dilation, hence raising the question of the true duration of the disease. Finally, we evidenced that venous macroaneurysms are a frequent consequence of longstanding RVO, whose identification by ICG angiography and subsequent photocoagulation may improve visual outcome. We will propose here a novel classification of retinal vein occlusions taking into account these features.

• 3415

**Discussion of clinical cases in real-life practice**

*MAUGET-FAYSSE M*

*Paris*

In real-life practice, in the light of the new concepts presented in this Special Interest Symposium, the management of retinal vein occlusion cases will involve:- The classification of venous occlusion, - The neovascular risk,- The presence of macroaneurysms- The general prognosis and treatment.Clinical cases concerning different types of venous occlusion will be presented to identify the cardinal signs for classification and treatment. In particular, we will present a case of central vein occlusion with peri venular whitening, a case with increased neovascular risk, a case with the cardinal signs for classification etc...At the end of the presentation, the audience will be able to recognize these cardinal features and the elements of the new classification.

• 3421

**What research tells us about the impact of glaucoma on the patient**

RAMILU P

*Wilmer Eye Institute, Johns Hopkins University, Baltimore*

Understanding the impact of glaucoma on the person is critical for treatment and public policy decisions. The functional domains impacted by glaucoma include mobility, driving, reading, and search-related tasks. A major impact of glaucoma is falls, and a one in three glaucoma patients experiences an injury resulting from a fall in a given year, likely as a result of balance impairment. The result is significantly more fear of falling than normally-sighted individuals, leading to significant restriction of physical activity, walking, and travel outside the home. Individuals with glaucoma are also more likely to stop or restrict their driving resulting in decreased independence, while those who continue to drive have higher rates of motor vehicle accidents. Glaucoma is associated with slower reading speeds, particularly when reading out loud, reading over longer durations, or reading low contrast materials. Finally, glaucoma affects a broad array of other abilities important in daily life including searching for objects, reaching and grasping objects, and recognizing individuals or objects. These impairments decrease quality of life for the individual and confer additional health risks, i.e. from fall or accident-related injury.

• 3422

**Methods used to measure patient perceptions**

RUBIN GS (1, 2)

(1) *UCL Institute of Ophthalmology, London*

(2) *NIHR Moorfields Biomedical Research Centre, London*

The number and popularity of patient-reported outcome measures (PROMs) have increased dramatically over the past two decades. At last count there were over 100 vision-specific PROMs covering all of the major eye diseases, including glaucoma. In this paper we discuss some of the main issues faced by developers and users of PROMs in glaucoma. Glaucoma-specific PROMs have been developed for many levels of patient assessment ranging from multifactorial "quality of life" questionnaires (e.g. the Glau-QoL and GQL-15) to the more specific symptom scales (GSS). The standard validation procedure for PROMs ensures that questionnaire scores are in fairly good agreement with measured performance of everyday visual tasks, at least for PROMs that are designed to measure activity limitation or functional ability. But they don't always agree, and we will illustrate how this disagreement may be informative and help identify patients who are at risk for functional decline. Finally, we will explore how modern questionnaire design, especially Rasch analysis, has changed the way we think about questionnaire scores and the implications of these changes for improving the content (item banks) and efficiency (computer adaptive testing) of future PROMs.

• 3423

**The art of healthcare: the importance and impact of effective Patient and Public Involvement (PPI)**

PORTEOUS C

*NIHR Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London*

Patient and Public Involvement (PPI) is a central component to health and social care research carried out in the UK, Europe, North America and Australia. It has a long history, from its roots in social activism and in patient movements through to clinical and political policy changes. However, PPI is yet to emerge as an important consideration in research and development within ophthalmology. The presentation will explore the some of the basics of patient and public involvement, including what it is, who it encompasses, and why PPI has an important role in research. The presentation will review the outcomes of the recent 'Glaucoma Day' held in London as an illustration of the importance of involving the public in research. Finally, the challenges and impact of PPI on eye research will be discussed

• 3424

**What glaucoma means to me: The patient's perspective**

KOTECHA A

*NIHR BRC for Ophthalmology, UCL Institute of Ophthalmology and Moorfields Eye Hospital NHS Foundation Trust, London*

The patient's perspective is becoming increasingly important within ophthalmology, with a wide variety of instruments available to 'measure' the patient's response and attitudes to their disease status, and the impact of new treatments on their well-being. The results of these are often used to facilitate change in how conditions are managed. However, they don't always fully capture the patient experience. This talk will consist of a series of short interviews with patients suffering with glaucoma to give a snapshot of what really matters to them, how they manage in every day life with their condition, what they think of their hospital visits and how they perceive the role of their caring clinician in the management of their disease.

• 3431

**Fitting tips and tricks of challenging cases of keratoconus**

ABOULSAMRA W  
ophthalmology, Mansoura

**Purpose** to discuss the fitting tips and tricks of challenging cases of keratoconus with a special design of RGP lens namely Rose K2 lens

**Methods** The study was carried on 52 keratoconic eyes fitted with Rose K2 lens. They were topographically classified into low cones, small steep cones, early cones, advanced cones and toric cones. The appropriate lens parameters were selected accordingly. Successful fitting was evaluated by lens position, movement, fluorescein pattern, visual acuity, residual astigmatism, patient comfort, maximum wearing time per day and corneal integrity.

**Results** The keratoconic eyes were successfully fitted as follow: lenses with bigger diameter and considerably flatter base curve than normal were used to fit the low cones, lenses with flatter base curve, minimum diameter and maximum edge lift were used in small steep cones. Keeping the lens as small as possible and considerably steeper base curve than normal were used in advanced cones. Early cones were fitted with larger diameter lenses with decreased edge lift. Toric or astigmatic corneas were fitted acceptably either by reducing the overall diameter of the lens or using toric RGP lens. With the previous lens parameters, most of patients (96%) tolerated their lenses with maximum wearing time per day 11± 2.5 hours. No corneal staining or abrasion was encountered in studied eyes. Lenses were centrally positioned with adequate movement and acceptable fluorescein pattern. Best corrected visual acuity was significantly increased compared to the baseline visual acuity with significantly decreased astigmatism.

**Conclusion** Patients with keratoconic corneas are sometimes challenging to fit and time consuming, but if well fitted with appropriate lens parameters, they appreciate the benefits provided by their contact lenses i

• 3432

**Current phenotype of keratoconus patients at King Khaled Eye Specialist Hospital**

AL-SWAILEM S (1), YILU S (2), AL ASSIRIA (1), ASGHARN (1), AL QASSIMIA (1)  
(1) King Khaled Eye Specialist Hospital, Riyadh  
(2) Wilmer Eye Institute, Johns Hopkins Medicine, Baltimore

**Purpose** To identify the characteristics of Keratoconus (KC) patients referred to the largest tertiary eye institute in Saudi Arabia (SA).

**Methods** Chart review of 483 patients from Jan. to Dec 2010. Amsler-Krumeich classification was used for severity staging.

**Results** The mean age of 944 eyes at diagnosis and referral were 21.6 and 28.1 years, respectively. Male to female ratio was 2:1. Mean central corneal thickness was 390 (range, 221-619) µm. Staging asymmetry between both eyes in bilateral (95.4%) cases were present in 53.8%. Male patients were diagnosed in mild stage (stage1), while, female patients in advanced one (stage 4). Advanced stage was significantly diagnosed at mean age of 19.76 years. Uncorrected visual acuity decreased with increasing disease staging ( $\geq 20/80$  in 46.4% of stage 1, while  $< 20/200$  in 39.1% of stage 4). Best corrected visual acuity, with either spectacles or contact lenses (CL), was  $\geq 20/40$  in 79.7% of eyes. Less eyes achieved 20/40 with spectacles in comparison to those fitted with CL. Single or combined management included: spectacles (15.4%), CL (57.6%), corneal collagen cross-linking (3.9 %), corneal rings (10.6%) and keratoplasties (39.3%). Corneal rings were required in mild (62%), moderate (35%), and advanced (3%) stages. Sub-epithelial and deep stromal scarring was present in 61.2% of eyes which required keratoplasties, and 2/3 of which were having moderate and advanced stages. Male patients tended significantly to have keratoplasty done at 20-30 years, while, female patients at >30 years of age.

**Conclusion** Our KC patients are similar to those reported in Indians with regards to early disease-onset, advanced stages, rapid progression, more scarring & lower contact lens wearers.

• 3433

**Corneal refractive power after anterior lamellar versus penetrating keratoplasty**

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(2) Ophthalmology, CHNO, Paris

**Purpose** To analyze the influence of various factors on the post-operative refractive power after anterior lamellar keratoplasty (ALK) and penetrating keratoplasty (PK) in eyes with corneal diseases not involving the corneal endothelium.

**Methods** Retrospective comparative case series. Two hundred and thirty-eight consecutive eyes of 238 patients with clear graft and at least one post-operative Orbscan examination performed after suture removal were retrospectively analyzed. Multivariate analysis of recipient and surgical parameters was performed by multivariate regression.

**Results** The average Orbscan 3-mm corneal power was 47.1 D with a wide 95%-confidence interval [40.3 D; 53.9 D]. It was 47.9 D for ALKs and 46.2 D for PKs ( $p<0.01$ ). In eyes with anterior lamellar keratoplasty, this figure was 48.2 D for oversized grafts and 44.0 D for non-oversized grafts ( $p<0.001$ ). It was significantly higher for eyes with keratoconus (48.2 D) or stromal scar after infectious keratitis (47.8 D) compared with stromal scar after trauma (43.6 D) or stromal dystrophies (41.3 D). The average Orbscan 3-mm SimK cylinder was 4.7 D [0.0 D; 10.7 D] for ALKs and 5.2 D [0.0 D; 10.9 D] for PKs ( $p>0.5$ ).

**Conclusion** The post-operative, suture-out, corneal refractive power is higher after ALK compared with PK and it features important variability. In ALK eyes, non-oversized grafts result in post-operative corneal power close to normal values and corneal diseases associated with stromal thinning result in higher post-operative corneal power. Taking into account these parameters at the time of surgery may help decreasing the post-operative spherical refractive error.

• 3434

**Recurrence of anterior corneal dystrophies after keratoplasty**

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**Purpose** To describe the rates of simple recurrence (SR) and clinically significant recurrence (CSR) of anterior dystrophies in donor corneas after penetrating or lamellar keratoplasty.

**Methods** Retrospective case series of patients who underwent keratoplasty for anterior dystrophies from 1954 to 2008 with a minimum follow-up time of three years. Kaplan Meier survival curves were calculated for both parameters (SR and CR) to demonstrate recurrence of the different dystrophies thru time.

**Results** A total of 109 eyes of 66 patients were included: 8 cases of Bowman's dystrophy (6 eyes with CDRB/Reis Bucklers and 2 eyes with CDTB/Theil Bhenke), 19 cases of CDG/Granular, 53 cases of CDL/Lattice and 29 cases of CDM/Macular. The median follow-up time for each kind of dystrophy varied from 75 months (6.25 years) to 180 months (15 years). The survival function for simple recurrence was significantly higher (376.2 months/31 years) for cases of CDM, as compared to those dystrophies related to the keratoepithelin gene : CDRB=177.6 months, CDL=168 months and CDG =89.2 months. Clinically significant recurrence (CR) was noted predominantly in cases of CDL and CDG

**Conclusion** Keratoplasty (whether penetrating or lamellar) is an effective treatment for cases of anterior dystrophies, when visual axis is affected or when other less invasive treatment modalities fail. The statistically significant difference in survival time of recurrence after keratoplasty for the different kinds of anterior dystrophies coincides with literature and may help us in prognosticating the best treatment option for each individual case. Theme: DALK and PK Does one or more of the authors have any financial interest in the subject matter of this presentation? No

• 3435

**Very low risk of light-induced retinal damage during Boston Keratoprosthesis surgery**

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(1) Cornea, Massachusetts Eye and Ear Infirmary, Boston

(2) Harvard Medical School, Boston

(3) Schepps Eye Research Institute, Massachusetts Eye and Ear Infirmary, Boston

**Purpose** To assess the possibility of light damage to the retina by a surgical microscope during implantation of a Boston Keratoprosthesis (B-KPro) in rabbits.

**Methods** The retinal irradiance from a Zeiss OPMI Lumera S7 operating microscope was measured at its working distance (16.5 cm). Light transmittance through an isolated B-KPro (made of PMMA) was measured. A B-KPro was implanted into one eye of each of 12 rabbits with the optic of the device initially covered during the procedure. Retinoscopy was performed after the implantation, and external additional lenses were used when needed to focus the light on the retina. The operated eyes were then continuously exposed to a fixed light intensity under the microscope for 1 hour. After the exposure, the rabbits were monitored for 9 days and fluorescein angiography (FA) was carried out at days 2 and 9.

**Results** Light transmittance of the B-KPro revealed blockage of short wavelengths (from 0.001% at 200nm to 42% at 390nm) and of long wavelengths (12-35% at 1660-1750nm). In addition, the surgical microscope blocked part of the blue, ultraviolet (UV), and infrared (IR) wavelengths of light. FA showed no leak of dye or other morphological changes. Histological examination with H&E staining showed no morphological retinal changes.

**Conclusion** Our results show that the PMMA of the B-KPro has a very low transmittance of phototoxic wavelengths of light, mostly UV. In addition, modern surgical microscopes have filters for UV and IR wavelengths of the light spectrum. No morphologic damage to the retina from the light exposure from our microscope could be demonstrated. Thus, there should be very low risk of light damage to the retina during routine B-KPro surgery.

• 3436

**Influence of growth factors on keratocyte phenotype in serum free media**

LYNCHA (1), O'SULLIVAN F (2), AHEARN E M (1)

(1) Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, Trinity College, Dublin

(2) NICB, Dublin City University, Dublin

**Purpose** Most studies examining the influence of growth factor on keratocytes in vitro have used serum supplemented media which promotes fibroblastic differentiation. The purpose of this study was to evaluate the effect of growth factors on the regulation of the keratocyte phenotype and extracellular matrix (ECM) production in vitro using a serum free media that has been shown to promote a keratocyte phenotype.

**Methods** Human keratocytes were cultured in keratocyte media (KM) consisting of Advanced DMEM, supplemented with GlutaMax, FGF-2 and L-ascorbic-2-phosphate. Cells were cultures for 14 days and supplemented with IGF-1, TGF $\beta$ -1 or TGF $\beta$ -3. DNA, sGAG and collagen synthesis were determined via biochemical assay. Expression of corneal, fibrotic and ECM related genes were examined by real-time PCR. ECM production was also examined by histological and immunofluorescent staining.

**Results** TGF $\beta$ -3 stimulated the highest level of proliferation and sGAG synthesis. In contrast, IGF-1 and TGF $\beta$ -1 showed significantly lower levels of DNA synthesis and sGAG production, comparable to KM alone. KM and TGF $\beta$ -3 showed the highest level of expression for keratanase. ECM and collagen production was evident for both the KM and TGF $\beta$ -3 group. The expression of  $\alpha$ SMA was greatest for TGF $\beta$ -3, while KDM demonstrated the highest expression levels for both collagen type I and III.

**Conclusion** These results demonstrate the influence different growth factors have on the phenotypic behaviour of keratocytes in serum free media. Further study in 3D culture will provide a useful model to further understand the effect growth factors have on keratocyte behaviour.

## Course 13: Don't panic: Hitchhiker's guide to statistics

### • 3441

#### Introduction to study design

DAMATO B

*Gayton - Wirral*

Much research is never published. The main cause of failure is the poor quality of the data, which does not allow valid conditions to be drawn. This problem can arise because of poor study design. The aims of this presentation are to discuss quality of data and the study designs developed to improve data validity. It is essential to be aware of the many sources of bias, which can fool the inexpert scientist at any stage of the project. These include selection bias, channelling bias, interview bias, chronology bias, recall bias, citation bias, confounding, and others. These shortcomings can be avoided by proper study design. Different levels of evidence have been defined to categorize study designs. These levels include: 'expert opinion without critical appraisal'; case-series; case control studies; cohort studies and randomized controlled trials, with systematic reviews in each category being superior to individual studies. Research should be undertaken only by individuals and teams who have a good understanding of study design. Adequate preparation before initiating the study should result in valid data and a good chance of publication in a high-impact journal, if the research findings are indeed new and useful.

### • 3442

#### Matching analysis with your study design

KIVELÄ T

*Department of Ophthalmology, Helsinki University Central Hospital, Helsinki*

This talk will highlight some key issues in choosing statistical methods for ophthalmic research, based on personal experience of the author as a writer, reviewer and editorial board member of the EVER Journal, *Acta Ophthalmologica*. The items discussed, based on examples from recent literature, will include: 1. proper ways of summarising and comparing visual acuities; 2. proper ways of analysing data collected from both eyes of the patients, 3. proper ways of analysing longitudinal follow-up data, and 4. proper ways of analysing contingency table data. Correct reporting and analysis of data related to the eye is not too difficult as long as one is aware of a few basic rules. Putting them into action helps to ensure that the conclusions you put forward and those that the readers draw from your study will be proper.

### • 3443

#### Communicating your research and presenting your data

DAMATO B

*Liverpool*

This presentation will focus on oral communications and scientific papers. An oral presentation must catch and hold the audience's attention, communicating the message unambiguously, succinctly, and memorably. Scientific papers must include all relevant methods and results, which must not only be honest but also convincing. The introduction must be sufficiently informative to educate the inexpert reader. The discussion must answer the most likely questions to be asked by the readership. Oral and written communications are most likely to succeed if they conform to conventional style. This talk will include tips and tricks for preparing effective oral presentations and scientific papers, with special attention being given to the presentation of data in the text, tables and figures. Useful sources of information will also be identified. It is tragic when important research is wasted because of poor communication. By adhering to simple rules, scientists can indeed bring their research projects to a successful conclusion, which is to disseminate new knowledge widely and effectively, by delivering inspirational presentations at influential conferences and publishing much-cited articles in high-impact journals.

### • 3444

#### Pearls and pitfalls

KIVELÄ T

*Department of Ophthalmology, Helsinki University Central Hospital, Helsinki*

The instructor will highlight some pearls and pitfalls in statistics that both authors and readers benefit from knowing, based on his personal experience as a writer, reviewer and editorial board member of the EVER Journal, *Acta Ophthalmologica*. The items discussed, based on examples from recent literature, will include: 1. how to avoid giving a false impression of precision when reporting key results, 2. how to communicate effect size; 3. how to differentiate statistical from clinical significance, 4. how to communicate statistically non-significant results, and 5. how to recognise multiple comparisons and adjust for them. Clear reporting of basic statistics is not difficult as long as one is aware of some basic rules. Putting them into action may improve your manuscripts and reading skills when scanning published evidence to base your practice on.

• 3451

**Retinal pigment epithelial (RPE) cells convert bone marrow derived macrophages into myeloid suppressor cells with novel phenotype**

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**Purpose** We have shown previously that macrophages/microglia accumulate in the subretinal space and express CD68 and Arginase-1 in the aging eye. We hypothesize that Retinal Pigment Epithelial (RPE) cells may play an important role in regulating macrophage/microglial phenotype and function.

**Methods** Bone marrow derived macrophages (BMDMs) and RPE were cultured from C57BL/6 mice and the phenotype was confirmed by CD11b and F4/80 (for BMDMs) and RPE65 and cytokeratin (for RPE cells) stainings. BMDMs were co-cultured with RPE cells for different times. Macrophages were then isolated for phenotypic and functional assays.

**Results** Co-culture of BMDMs with RPE cells resulted in a time-dependent down-regulation of MHC-II and the generation of CD11b<sup>+</sup>F4/80<sup>+</sup>Ly6G<sup>+</sup> myeloid-derived suppressor cells (MDSC). The MDSCs expressed high levels of IL-6, IL-1 $\beta$ , Arginase-1, and complement inhibitor C1INH, but lower levels of IL-12p40 and TNF- $\alpha$  compared to naïve BMDMs. The expression levels of iNOS, TGF- $\beta$  and Ym1 did not change compared to naïve BMDMs. Furthermore, MDSCs had reduced phagocytic activity and lower ability to stimulate T cell activation and proliferation. When RPE cells were pre-treated with oxidized photoreceptor outer segments, the expression of IL-1 $\beta$  and IL-6 in BMDMs was increased and the expression of Arginase-1 was decreased.

**Conclusion** Our results suggest that healthy RPE cells can convert BMDMs into myeloid-derived suppressor cells under in vitro culture conditions. RPE-induced myeloid-derived suppressor cells are CD11b<sup>+</sup>F4/80<sup>+</sup>Ly6G<sup>+</sup>MHC-IIlowIL-6+IL-1 $\beta$ +Arg-1<sup>+</sup>. This ability of RPE cells is reduced when suffering from oxidative insults.

• 3452

**Ocular Syphilis: Beware of the great imitator!**

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(1) Ophthalmology, Enschede

(2) Ophthalmology, VUmc, Amsterdam

(3) Microbiology, Enschede

(4) Internal Medicine, Enschede

**Purpose** Treatment of HIV with highly active antiretroviral therapy (HAART) has extended life expectancy with many years. There are signs that the better prognosis of this formerly deadly disease has a negative spin-off, which is a frequent occurrence of the coexistence of HIV and ocular syphilis.

**Methods** We collected the cases of ocular syphilis in the last 7 years in a general hospital ( Medisch Spectrum Twente, the Netherlands). Furthermore, a literature search was performed to identify papers on the clinical manifestation of this disease, especially in relation to HIV-infection.

**Results** We analysed the characteristics of 3 patients with ocular syphilis, 2 of these patients had co-infection with HIV. Several different disease manifestations of *Treponema Pallidum* infection in the eye were observed, especially with regard to the optic nerve, the macular area and the retinal periphery. Treatment with intravenous Penicillin was successful in all patients, though some eyes developed irreversible loss of function.

**Conclusion** Especially in immune compromised patients ocular syphilis is an acute sight threatening disease, with specific diagnostic challenges, requiring immediate intravenous treatment.

• 3453

**Intraocular rubella virus detection as a diagnostic tool for atypical forms of Fuchs cyclitis**

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LABETOUILLE M (2), ROZENBERG F (1), BODAGHI B (1)

(1) CHU Pitié-Salpêtrière, Paris

(2) CHU Bicêtre, Kremlin-Bicêtre

**Purpose** Since 2004, different studies have reported on the pathophysiology of Fuchs cyclitis, which may be due to rubella virus infection. The purpose of this study is to investigate clinical profile and prognosis of rubella virus associated uveitis. Retrospective, observational, bicentric study.

**Methods** All patients managed between January 2009 & march 2013 for an atypical form of Fuchs cyclitis were included. Clinical features for these patients were assessed. An extensive work-up of anterior or intermediate uveitis was performed in all cases. An anterior chamber tap was done for molecular detection of herpesviruses and intraocular anti-rubella virus antibody synthesis. Treatment was adapted to the results of ocular fluids analysis.

**Results** The series included 17 patients (M/F: 11/6) with a proven diagnosis of rubella virus-associated uveitis. The mean age was 43.3 years (27-70). All patients had a positive outcome for intraocular antibody production against rubella virus with a Goldmann-Witmer coefficient > 3. Only 3 patients (15%) fulfilled the classical Fuchs cyclitis criteria with stellar keratic precipitates + heterochromia/iris atrophy + cataract + vitritis. Uveitis was bilateral in 3 patients (20%) and 7 patients (35%) had an uncontrolled IOP requiring glaucoma surgery.

**Conclusion** For most of these patients, intraocular rubella virus serology allowed the final diagnosis of Fuchs cyclitis despite incomplete clinical criteria. Intraocular rubella virus detection can help for the diagnosis of atypical forms of Fuchs cyclitis. This avoids unnecessary additional tests and prescription of local or systemic corticosteroids. Secondary glaucoma remains a major complication and needs a close monitoring.

• 3454

**Mid-term efficacy and safety of adalimumab in refractory pediatric uveitis : A retrospective monocentric study**

PENAUD B

Ophthalmology department, Pitié-Salpêtrière hospital, Paris

**Purpose** Evaluate the mid-term efficacy and safety of adalimumab in the pediatric population with severe uveitis.

**Methods** We retrospectively analysed 18 children with severe uveitis, who were treated with adalimumab (20 or 40 mg every two weeks) when the previous immunosuppressive therapy has been ineffective. It consisted of systemic corticosteroids (n=18), methotrexate (n=18), azathioprine (n=8), infliximab (n=6), etanercept (n=6), interferon-alpha (n=1). Primary outcome was to assess laser flare photometry values after adalimumab therapy compared with baseline. Clinical features (SUN criteria, macular edema and papillitis), oral prednisone threshold, visual acuity, side effects and complications of treatment were also considered.

**Results** Median age was 13.1 years (range 6-20.8), sex ratio (F/M) was 3. Median duration before adalimumab therapy was 82.6 months (range 16-262). The mean follow-up was 35.5 months (range 23-63) and the final median laser flare photometry value was significantly reduced from 149.5 ph/ms (range 24-335) to 81.3 ph/ms (range 4-224) p<0.005. Median oral prednisone decreased from 10.3 mg/day (range 0-30) to 1.7 mg/day (range 0-15) p<0.05. Uveitis was controlled in 10 cases (55.5%). Relapses occurred in 2 cases (11%), adalimumab was ineffective in 3 cases (17%) and was stopped in one patient who had excellent control of inflammation. Three children (17%) discontinued treatment due to severe side effects.

**Conclusion** Adalimumab appears to be an effective and well tolerated treatment for refractory pediatric uveitis, with prolonged control of inflammation over several years, even after failure of other anti-TNF alpha agents. A prospective randomized double blind study is ongoing.

**• 3455****Tocilizumab for uveitic macular edema**

MESQUIDA M, LLORENÇ V, ADANA A

Ophthalmology, Barcelona

**Purpose** To report the outcomes of tocilizumab (TCZ) treatment for uveitic cystoid macular edema (UCME) refractory to immunomodulatory therapy.

**Methods** Five refractory patients with UCME who received TCZ between January and August 2012 were identified by retrospective chart review. All patients received 8 mg/kg TCZ at 4 week intervals. Data regarding patient demographics, use of immunosuppressive (IS) drugs, biologic agents or intravitreal therapies prior to TCZ infusions were gathered. Main outcome measures: Central foveal thickness (CFT) measured by optical coherence tomography, degree of anterior and posterior chamber inflammation (Standardization of Uveitis Nomenclature Working Group criteria), and visual acuity (logarithm of the minimum angle of resolution [log-MAR]) were recorded at month 1, 3, and 6.

**Results** Eight eyes from 5 patients (all females) were included. Mean age was 49.4 years (range, 30-68). Mean follow-up was 8.4 months (range, 6-12). Before TCZ, all patients received and failed conventional IS therapy and had received at least 1 another biologic agent. Uveitis diagnoses were: Birdshot chorioretinopathy (n=3), juvenile idiopathic arthritis-associated uveitis (n=1), and idiopathic panuveitis (n=1). Mean baseline CFT (95% confidence interval) was  $602 \pm 236 \mu\text{m}$  in baseline,  $386 \pm 113 \mu\text{m}$  at month 1 ( $p=0.006$ ),  $323 \pm 103 \mu\text{m}$  at month 3 ( $p=0.026$ ) and  $294.5 \pm 94.5 \mu\text{m}$  at month 6 ( $p=0.014$ ). Median log-MAR best-corrected visual acuity (BCVA) improved from  $0.66 \pm 0.57$  in baseline to  $0.47 \pm 0.62$  at month 6 ( $p = 0.035$ ). After 6 months, an improvement of  $\geq 2$  lines of BCVA was observed in 50% of eyes ( $p=0.028$ ). Sustained uveitis remission was achieved in all patients. No AE were reported.

**Conclusion** These data suggest that TCZ is effective for treating UCME in otherwise refractory cases.

**• 3456****3 months results of intravitreal dexamethasone implant in non infectious uveitis**

DARUGARA, CHAMPION E, TOUITOU V, FARDEAU C, LE HOANG P, BODAGHIB

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**Purpose** To evaluate efficacy and safety of dexamethasone implant in patients with non infectious uveitis.

**Methods** Retrospective study. Charts of 30 patients with non infectious uveitis treated with dexamethasone implant between September 2012 from April 2013 were reviewed. Best corrected visual acuity (BCVA), intra-ocular pressure (IOP), laser flare meter and retinal central mean thickness (CMT) were followed up initially, at 1 and 3 months in each patient.

**Results** 29 patients of 30 were treated for a macular edema. 1 patient was treated to improve the anti-inflammatory control for a Behcet disease refractory to immunosuppressive therapy. Intravitreal dexamethasone injection was effective in improving BCVA : preoperative mean BCVA was 0.57 logMAR units and improved to 0.45 and 0.43 logMAR units at month 1 and 3. Mean CMT also improved in all patients : the pre-operative mean CMT was  $455 \mu\text{m}$  and improved to 300 and  $312 \mu\text{m}$  at months 1 and 3, respectively. Macular edema began to relapse in 23 patients at 3 months. Mean anterior chamber flare measured by laser decreased from 250 photons/ms to 60 photons/ms. Safety was good with 3 cases of IOP increase resolved by medical treatment only, 1 case of intravitreal hemorrhage treated by vitrectomy.

**Conclusion** OZURDEX<sup>(\*)</sup> appeared effective in management of uveitis macular edema but its action was short with frequent relapse at 3 months. Its safety seemed to be good. Further studies are needed to evaluate its place in the treatment strategy.

• 3461

**Three-dimensional analysis of HSV colour space to distinguish small retinal haemorrhages, hard exudates and photocoagulation marks from dust artefacts**

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(2) *Ophthalmology and Visual Science, Hiroshima University, Hiroshima*

**Purpose** To detect early diabetic retinopathy, we employed three-dimensional analysis of HSV colour spaces.

**Methods** Six patients with diabetic retinopathy and small haemorrhages, hard exudates and photocoagulation marks were evaluated using fundus photography, which revealed dust artefacts in the fundi of some patients. We constructed an experimental device similar to the optical system of a fundus camera and made the artificial eyes of the fundus painted four kinds of colours: khaki, sunset, rose and sunflower. The dust artefacts were photographed under each artificial eye using the experimental device. We analysed all images using Scilab 5.4.0 and SIVP 0.5.3 software, which changed the RGB colour space into an HSV colour space. The software interpreted each value of HSV colour space as a three-dimensional graph, which was modified using a Gaussian filter.

**Results** We calculated the difference between manifestation and perimannifestation areas and dust artefact and periartefact areas using average HSV values. V values for the manifestations were haemorrhage,  $0.06 \pm 0.03$ ; hard exudate,  $-0.12 \pm 0.06$  and photocoagulation marks,  $0.07 \pm 0.02$ . V values for the dust artefacts, visualized under the human eye and the artificial eyes, were human eye,  $0.19 \pm 0.03$ ; khaki,  $0.41 \pm 0.02$ ; sunset,  $0.43 \pm 0.04$ ; rose,  $0.47 \pm 0.11$  and sunflower,  $0.59 \pm 0.07$ . Ratios of the dust artefact to manifestation areas in the human eye were haemorrhage, 3.17; hard exudate, -1.58 and photocoagulation, 2.71.

**Conclusion** The HSV colour space displayed good capability to distinguish small retinal haemorrhages, hard exudates and photocoagulation marks from dust artefacts.

• 3462

**Reliability of the cone counts by an Adaptive Optics Retinal Camera**

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(2) *Ophthalmology, Salpêtrière, Paris*

(3) *Methodology Center, Besançon*

**Purpose** To report the reproducibility and the repeatability of cone imaging in healthy human eyes, using the RTx-1™ Adaptive Optics Retinal Camera, (Imagine Eyes, Orsay, France) and AOdetect v0.1 cone counting software.

**Methods** Cones were imaged in healthy emmetropic adults. Mean cone density (cells/mm<sup>2</sup>), spacing between cells ( $\mu$ ) and percentage of hexagonal cells calculated on Voronoi diagrams, were measured with the proprietary software of the device, from a 0.3° square, positioned on a horizontal line crossing the fovea. The square was moved horizontally following a 10 $\mu$  step, until reaching the parafoveal density peak. Intersession reproducibility was assessed by imaging 3 subjects, 5 consecutive day. Intrassession repeatability was assessed by comparing 10 acquisitions of 3 subjects, in a row. Inter-operator reproducibility was evaluated by comparing 10 subjects imaged by 2 operators. Inter grader reproducibility was evaluated by assessing 60 different images by 2 masked graders. Correlation coefficient and coefficients of variation(COVs) were calculated.

**Results** The mean cone counts was respectively  $23623 \pm 3831/\text{mm}^2$  and  $22851 \pm 3543/\text{mm}^2$  for the first and the second operator (*t* test,  $p < 0.05$ ). Coefficient correlation was 0.81 between operators, and 0.97 between the 2 graders. The intrasession and intersession COVs were under 7%. The percentage of hexagonal cells and the spacing, varied in the same proportion (COV range from 1.66 to 10.05%).

**Conclusion** Cone counts using the RTx-1™ device and its software are reliable in normal human eyes. Further studies in the normal clinical setting are needed.

• 3463

**Quantification of cone loss after macula-involving retinal detachment using the RTX-1 Adaptive optics camera**

FLORES M, DEBELLEMANIERE G, MEILLAT M, TUMAHAI P, SCHWARTZ C, MONTARD M, DELBOSC B, SALEH M

*Service d'ophtalmologie CHU J. Minjoz, Besançon*

**Purpose** To image the macular cone photoreceptors in eyes with successful repair of retinal detachment (RD) and in healthy fellow eyes using the RTX-1 Adaptive optics camera from Imagine Eyes™ and to correlate the results to clinical outcomes.

**Methods** Patients operated for macula-off RD were imaged 6 weeks after surgery. The images were analyzed using AOdetect v0.1. Cone density, spacing between cells, and the nearest-neighbor analysis were measured 1° nasally and temporally from the fovea. Best corrected Visual acuity(BCVA) and thickness of the photoreceptor inner and outer segment junction (IS/OS) line imaged by SD-OCT were also measured.

**Results** Twenty one patients (42 eyes) were studied. The parafoveal cone density was decreased in eyes operated for RD (mean $\pm$ SD:  $14,576 \pm 4035/\text{mm}^2$ ) compared to fellow eyes ( $20,589 \pm 2350/\text{mm}^2$ ) (Wilcoxon matched pairs test,  $p=0.0001$ ). There was also an increase in cone spacing ( $10.3 \pm 2.6$  vs  $8.0 \pm 1.0$   $\mu\text{m}$ ,  $p<0.0001$ ). The Voronoi analysis revealed a reduction in the percentage of cones with six neighbors ( $36.5 \pm 4.2$  vs.  $42.7 \pm 4.6\%$ ,  $p=0.0003$ ). The IS/OS line thickness was correlated to the cone density in the same area ( $r=0.62$ ,  $p<0.0001$ ). BCVA was significantly correlated to cone density and inversely to intercone spacing, and in a lesser proportion to the percentage of cones with six neighbors ( $r=0.8$ ,  $-0.71$  and  $0.49$ , respectively,  $p<0.001$ ).

**Conclusion** There was a decrease in the cone density after RD with an estimated loss of one-third of the cones in comparison with fellow eyes. Visual acuity was highly correlated with the parafoveal cone density and cone spacing. AO may be a valuable prognostic tool after RD surgery.

• 3464

**Evaluation of cone photoreceptor density using adaptive optics retinal imaging in patients under treatment with hydroxychloroquine**

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*Service d'Ophtalmologie CHU Besançon, Besançon*

**Purpose** Hydroxychloroquine is a common treatment of many inflammatory diseases and is sometimes responsible of irreversible macular toxicity. Early screening is essential. Our study aimed to evaluate feasibility of adaptive optics imaging in this population and to measure parafoveal cone density.

**Methods** Patients under treatment or consulting for a pre-treatment checkup were included. A complete ophthalmologic examination was performed, as well as a high-definition fundus photography using adaptive optics retinal camera rtx-1™ (Imagine Eyes, Orsay, France). Cumulative dose of hydroxychloroquine was calculated. Parafoveal cone density, cellular spacing, and percentage of hexagonal cells calculated using Voronoi diagrams were calculated using AOdetect v0.1 software (Imagine Eyes, Orsay, France).

**Results** Sixty eyes of 30 patients (27 females, 3 males) were imaged. There was a significant correlation between maximal parafoveal cone density and cumulative dose of hydroxychloroquine. ( $R^2 = 0.20$ ,  $p = 0.029$ ). This relation was also found with cellular spacing ( $R^2 : 0.2$ ,  $p = 0.03$ ) and hexagonal cells percentage estimated on Voronoi diagram ( $R^2 : 0.19$ ,  $p = 0.03$ ). By contrast, no statistically significant difference was found by comparing the group "cumulative dose < 1000g" with the group "cumulative dose > 1000g" (Mann-Whitney test,  $p = 0.14$ ).

**Conclusion** Parafoveal cone density measurement using adaptive optics retinal camera is achievable in this population. Our study seems to highlight a relation between cumulative dose and parafoveal cone density. Age and axial length are possible confounding factors that could not be studied in this study.

• 3465

**Comparison of macular pigment optical density measured by autofluorescence and reflectometry: The LIMPIA study**

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(4) Univ. Bordeaux, ISPED, Centre INSERM U897-Epidemiologie-Biostatistique, Bordeaux

(5) INSERM, ISPED, Centre INSERM U897-Epidemiologie-Biostatistique, Bordeaux

**Purpose** To compare macular pigment optical density (MPOD) measured by two techniques (autofluorescence, reflectometry) in healthy subjects at high risk for age-related macular degeneration (AMD).

**Methods** The Limpia Study is a double-blind, placebo controlled, prospective randomized clinical trial performed in 120 subjects with at least one parent affected by neovascular AMD. Subjects having used supplements containing lutein and/or zeaxanthin in the preceding year were not included. MPOD was measured using two methods: the two-wavelength autofluorescence method with a modified scanning laser ophthalmoscope (Heidelberg Retinal Analyzer

**Results** At baseline, mean MPOD within 0.51°, measured with the modified HRA method, was 0.5 (standard deviation 0.2). Maximal MPOD, measured with Visucam, was 0.4 (standard deviation 0.1). Parameters from the modified HRA (optical density within 0.5°, 1°, 2° and 6°) and from the Visucam method (volume, area, mean and maximum optical density) were weakly correlated. The best correlations were observed for MPOD within 2° and 6° with volume from Visucam ( $r=0.21$  and  $r=0.22$ , respectively), and mean MPOD from Visucam ( $r=0.21$  and  $r=0.18$ ).

**Conclusion** The two methods propose different parameters to evaluate macular pigment, which overall correlated weakly in this population of middle-aged healthy subjects at high risk for AMD. Further research is needed to characterize the differences between the methods and identify the best parameters and techniques to measure macular pigment.

*Commercial interest*

• 3467 / F057

**Evaluation of ultra-widefield fundusautofluorescence in non-infectious posterior uveitis**

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**Purpose** Posterior uveitis comprises a heterogenous group of diseases with inflammatory alterations of the posterior fundus and is a common cause of visual impairment and blindness. The goal of this study is to evaluate the diagnostic value of wide-field-fundusautofluorescence (FAF) of patients with non-infectious posterior uveitis and chorioretinal alterations.

**Methods** 73 eyes from 51 patients were included. Best-corrected visual acuity, wide-field color and FAF images achieved by a wide-field SLO (Optomap P200Tx, Optos PLC, Dunfermline, Fife, Scotland, UK) and a full ophthalmological examination were obtained from each patient. A systematic analysis of chorioretinal alterations detected with FAF and color images was conducted followed by the evaluation of the diagnostic information of wide-field FAF compared to the clinical finding and wide-field color images respectively.

**Results** 52 of 73 included cases showed peripheral alterations. In 32 cases, wide-field FAF images revealed a greater number and more extended chorioretinal alterations than corresponding wide-field color images of the posterior fundus.

**Conclusion** In this study, wide-field FAF images showed more chorioretinal alterations than seen in funduscopic or in color SLO images. Therefore, wide-field FAF images offer important additional information in detection and documentation of peripheral and central chorioretinal alterations.

• 3466 / F041

**Autofluorescence patterns and visual acuity in macular telangiectasia type 2**

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(2) EMMES Corporation, Rockville, Maryland

**Purpose** An analysis was performed with a view of determining potential associations between typical patterns seen on autofluorescence (AF) imaging in patients with Macular Telangiectasia (MacTel) type 2 and distance visual acuity at presentation as well as visual change over a 2-year follow-up period, thus ascribing predictive value to AF patterns in MacTel.

**Methods** A subgroup of 135 patients (229 eyes) enrolled in the MacTel study underwent AF imaging. Images were graded at the Moorfields Eye Hospital Reading Centre. Recorded AF patterns at baseline and at two years included the typical pattern of increased central AF due to loss of foveal masking, localized decreased AF at the end of a retinal vessel and a large area of decreased AF. Best-corrected visual acuity (BCVA) was measured by means of the Early Treatment for Diabetic Retinopathy Study charts at baseline and after two years. Statistical associations were derived by means of a generalized linear model.

**Results** Presence of increased macular AF ( $p=0.004$ ), a large area of decreased AF ( $p<0.001$ ) or decreased AF at the end of a retinal vessel ( $p<0.001$ ) at baseline were significantly associated with worse BCVA. Presence of increased macular AF ( $p<0.001$ ) or of localized decreased AF at the end of a retinal vessel ( $p<0.001$ ) and the absence of a large area of decreased AF ( $p<0.001$ ) were predictive of a subtle, but statistically significant drop in BCVA at 2 years.

**Conclusion** The typical pattern of increased central AF at baseline is encountered in patients with worse BCVA in MacTel type 2 and is also predictive of further subtle visual loss in a period of two years. Decreased AF is found in patients with worse baseline BCVA though no further visual loss at two years is observed.

• 3468

**The effect of posterior subtenon injection of triamcinolone acetonide for diabetic macular edema refractory to intravitreal bevacizumab injection**

SOO GELIN JOE (1), MOON HAEIN (1), CHAE JB (2)

(1) Ophthalmology, Seoul

(2) Ophthalmology, Cheongju

**Purpose** To evaluate posterior subtenon injection of triamcinolone acetonide (stTA) for diabetic macular edema (DME) refractory to intravitreal bevacizumab injection.

**Methods** Patients with DME involving fovea who revealed central foveal thickness (CFT) over than 300 $\mu$ m continuously and did not respond to intravitreal bevacizumab injection (IVB) were included. When CFT increased after 1 or 2 IVB or CFT did not decreased more than 50 $\mu$ m after 3 consecutive IVB, we classified these group of patients as DME refractory to IVB. Other diseases which can cause macular edema including macular degeneration, vein occlusion and epiretinal membrane were also excluded. 40mg TA was injected in to the posterior subtenon space by one clinician. All patients received ophthalmic examination including Snellen visual acuity, intraocular pressure (IOP), spectral domain optical coherence tomography at the time of posterior stTA injection, 2-month, 4-month and 6-month follow up period.

**Results** 41 eyes of 35 patients were included. Average CFT reduced from 474 $\mu$ m to 377 $\mu$ m at 2-month, to 352 $\mu$ m at 4-month and 401 $\mu$ m at 6-month ( $p<0.001$ ,  $p=0.001$ ,  $p=0.035$  respectively; paired T-test). Average IOP were increased from 15.5mmHg to 16.9mmHg at 2-month but it reduced to 16.2 at 4-month and to 15.8 at 6-month. Visual acuity in logMAR scale increased from 0.55 to 0.50 at 2-month, to 0.49 at 4-month and to 0.50 at 6-month ( $p=0.001$ ,  $p<0.001$ ,  $p<0.001$  respectively; paired T-test). No complications such as glaucoma and cataract were detected.

**Conclusion** Posterior stTA is effective treatment for DME refractory to IVB. But the effect did not continue over 6 months.

• 3471

### Anatomy of afferent visual pathway

KAWASAKIA  
Lausanne

The afferent visual pathway encodes and transmits all aspects of image perception from retina to occipital lobe. A critical feature of this pathway is the topographic organization of the fibers that make up the afferent visual pathway. Knowledge of the anatomy and topography of the afferent visual pathway will aid recognition and localization of specific patterns of visual field defects.

• 3472

### Principles of automated perimetry

SZATMARY G  
Hattiesburg

**Principles of Automated Perimetry: Types and Uses**

**Background:** Visual field testing is sensitive and has localizing value, therefore is an essentially part of the clinical evaluation of every patient with complaint related to the afferent visual system.

**Objective:** To review the most informative available kinetic and static visual field methods.

**Indication:** basic principles, advantages and disadvantages of the various methods will be discussed.

**Methods:** Retrospective review of the literature on perimetry methods presented along with representative cases.

**Results:** Apart from availability, indication that is based on clinical suspicion, should determine the applied perimetry method in a particular patient's evaluation.

**Sensitivity of visual field testing** supersedes most other available diagnostic methods even magnetic resonance imaging.

**Conclusion:** Familiarity with broad range of perimetry methods is essential in the initial clinical assessment and follow up care of patients with afferent visual system complaints.

• 3473

### How to perform confrontational visual fields, tangent screen and manual kinetic perimetry

KAWASAKIA, BORRUAT FX  
Lausanne

Non-automated visual field testing remains an important clinical skill. Confrontational testing is an important bedside test that can detect subtle hemianopic defects, particularly when using a red test object. Confrontational and kinetic perimetry are also excellent ways to document a non-organic origin of severely constricted visual fields. Tangent screen testing and kinetic perimetry are capable of identifying small central defects, particularly those missed on standard automated threshold tests and can delineate the pattern of defect for purposes of localization. This lecture will review and provide tips on how to perform and interpret these non-automated forms of perimetry in the appropriate clinical situation.

• 3474

### Cases and visual field examples and discussion

KAWASAKIA (1), BORRUAT FX (1), SZATMARY G (2)

(1) Lausanne  
(2) Hattiesburg

cases for audience

**Industry-sponsored symposium 3 : Enhanced Oxidative Stress and ocular surface**

• 3521

**Reduction of oxidative stress: Importance for age-related diseases**

*GARHOFER G  
Vienna*

**ABSTRACT NOT PROVIDED**

• 3522

**Clinical results on the anti-oxidative effect of oral vitamin and mineral supplementation (on impaired retinal vascular reactivity)**

*GARHOFER G  
Vienna*

**ABSTRACT NOT PROVIDED**

• 3611

### Mechanism of fluid accumulation

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Purpose: To characterise the cellular mechanisms of fluid flow in the retina in view of its clinical implications. Background: Retinal fluid homeostasis is effected by a whole host of mechanisms that reside both in the retinal pigment epithelium, in the choroid and in the retina. In a physiological state there is a constant flow of fluid out of the intraretinal and subretinal space into the choroid, of which about 70% is effected via active transport mechanisms within in the retinal pigment epithelium. In case these Results: This presentation will summarise the latest data on how fluid homeostasis is maintained within the retina and how these mechanisms underly the evolving pharmacological treatment modalities for exudative maculopathies. Conclusion: The understanding of the cellular mechanisms that govern retinal fluid flow is crucial to the clinical application of medical treatments against exudative maculopathies.

• 3612

### Fluid of choroidal origin

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*Hotel Dieu de Paris University Descartes, Paris*

The choroidal circulation is crucial in supplying nutrients as well as oxygen to the outer retina. The capillaries of the choriocapillaris are fenestrated and have a high protein permeability that allows the establishment of a high oncotic pressure, presumably contributing to movement of fluid out of the retina through the stroma to the sclera. The pores of the choriocapillaris are facing the RPE. Bruchs membrane acts as a sieve depending on the amount and chemical properties of the deposited waste products. The accumulation of fluid under the RPE or within the retina implies a tight Bruchs membrane. Lipids as result of the outer segment turn over accumulate and decrease the permeability of Bruchs membrane precluding the evacuation of the waste products and initiate a vicious circle. Active transport by the RPE is impaired. The tight junctions of the RPE are ruptured, the fluid irrigation into the retina results in the disorganisation of the outer layers of the retina. A progressive breakdown of all defense mechanisms especially the reabsorption pump tips over the balance. The mechanisms of the restoration are partially known. All choroidal diseases can induce these features and clinicians may analyze the resulting steps

• 3613

### Fluid from the retinal origin

*WOLF-SCHNURRBUSCH U*

Macular edema is a nonspecific but very common final pathway for many ocular diseases and not a specific entity. Related ocular disorders include diabetic retinopathy, vascular occlusions, post surgical situations and inherited disorders. The retinal fluid accumulation could be intracellular or an extracellular. Extra cellular fluid accumulation, which is more frequent and more clinically evident, is directly associated with an alteration of the blood-retinal barrier (BRB). Intracellular fluid accumulation leads to a cellular damage because of cytotoxic alterations of the cellular ionic distribution. These mechanisms may be complicated by the release of various cytokines, significant inflammation and/or ischemic processes. The influence fluid from the retinal origin should be illustrated with various diseases. Examples for typical findings of diagnostic tools like fluorescein angiography (FA) and optical coherence tomography (OCT) were given for each entity and described. We will discuss the different mechanisms of occurrence, the various clinical forms of fluid accumulation and the mechanisms of action.

• 3614

### Anti VEGF: When, which effect?

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Fluid accumulation in the retina is a multi-factorial process that involves the breakdown of the blood-retina barrier in retinal capillaries or the retinal pigment epithelium. Hydrostatic pressure gradient and the colloid osmotic pressure gradient lead to diffusion of fluid across capillary walls. Damage to tight junctions enhances the rate of capillary filtration, which exceeds the rate of fluid removal from the retina. Fluid accumulates in the outer plexiform layer and pools into cystic space. Accumulated fluid is visible on optical coherence tomography and fluorescein angiogram, but unlike clinical edema, its finding on imaging studies does not always correlate with changes in visual acuity. For that reason, periodical follow-up is required. Decision to initiate treatment or retreatment to prevent progression to edema with subsequent deterioration of visual acuity may be difficult. Intravitreal injections with anti-VEGF agents have become the first-line treatment for macular edema. Combination therapy with corticosteroids may be successful in chronic edema that has been found to be somewhat resistant to treatment. Early treatment for macular edema was shown to be beneficial for optimal visual outcomes.

*Commercial interest*

**SIS: Fluid accumulation in the retina****• 3615****Steroids: How does it work?***BEHAR-COHEN F (1, 2)**(1) Université Paris Descartes, Hôpital Sainte-Justine, Paris, France**(2) Inserm UMR S 872, Paris, France*

Glucocorticosteroids (GCs) are widely used to reduce macular edema of any origin. GCs induce a quick (within hours) reduction in macular thickness, not well correlated to vascular leakage. Kinetic of action suggests that GCs are involved in fluid elimination from the retina but their mechanisms of action remain poorly understood. GCs act through activation of gluco and mineralocorticoid receptors that are co-expressed in retinal cells such as retinal glial Muller cells and retinal pigment epithelial cells, both involved in hydro-ionic balance in the retina. Through binding to either gluco and/or mineralocorticoid receptors, GCs exert a direct regulation of ions and water movement through expression and distribution regulation of Kir 4.1, ENaC and AQP4 channels in retinal glial Muller cells. Depending on their differential affinity for gluco or mineralocorticoid receptors and depending on the dose, different GCs act differently on hydro ionic regulation. These findings open new insight in the mechanisms of action of GCs on retinal edema and suggest that a more rationale use of GCs could help optimize the ratio efficacy/side-effects of GCs.

**SIS: Retinal ganglion cell degeneration and dysfunction in glaucoma****• 3621****Retinal ganglion cell degeneration: events in the human retina**

*THANOS S  
Munster*

**ABSTRACT NOT PROVIDED****• 3622****Retinal ganglion cell degeneration and dysfunction in glaucoma**

*LELING C  
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Both dendritic shrinkage and dendritic outgrowth have been reported after chronic elevation of intraocular pressure and the longitudinal profile of retinal ganglion cell degeneration in glaucoma remains largely obscured. Monitoring dendritic and axonal degeneration requires long-term, *in vivo* examination of axon and dendritic arborization. While clinical imaging only permits visualization of the ganglion cell layer and the nerve fiber layer, animal models have been established to track axonal and dendritic changes. Using a confocal scanning laser ophthalmoscope to image the retinae of transgenic mice (Thy-1 YFP) that express fluorescent protein in neurons under the control of a Thy-1 promoter, retinal ganglion cell degeneration has been shown to begin with progressive dendritic shrinkage, followed by loss of the axon and then the cell body after optic nerve crush. Although challenges remain to develop a glaucoma model with mildly to moderately elevated intraocular pressure lasting for a sufficiently long period of time with a clear optical media, it is promising that *in vivo* analysis of dendritic arborization would provide new insights into the neurobiology of retinal ganglion degeneration and neuroprotection in glaucoma.

**• 3623****Psychophysical effects of RGC damage: What tests could we use clinically?**

*REDMOND T  
Cardiff University, Cardiff*

Visual field sensitivity is measured clinically in the investigation of retinal conditions such as glaucoma. It is also the primary functional endpoint in many clinical trials in ophthalmology. Since the introduction of the gold standard visual field test, Static Automated Perimetry (SAP), over 30 years ago, a great deal more has been learned about biological changes in the retina in eye disease, particularly in the retinal ganglion cells (RGCs) from cat, primate and rodent models, with many recent studies pointing towards pre-morbid degenerative events in these cells. There is also growing psychophysical evidence of enlarged RGC receptive fields in conditions such as glaucoma, before detection of damage by SAP. This has renewed interest in the design of functional tests that are capable of detecting subtle changes in the retina in early disease, in the hope that early visual loss can be halted or even reversed. The optimum visual field test is one that is sensitive to pre-morbid biological changes in the retina with minimal measurement variability and a large measurement range. This test should be applicable to a wide range of diseases that are characterised by RGC damage.

**• 3624****RGC degenerations. A neural substrate for reversing glaucomatous damage?**

*MORGAN J  
Cardiff*

The demonstration of retinal ganglion cell (RGC) dendritic remodelling and pruning in glaucoma raises the possibility that this could provide a neural substrate for the recovery of retinal ganglion cells. The associated reduction in the size of the retinal ganglion cell receptive field can arise as a result of the loss of dendrites but also due to the loss of synaptic connections. Since RGCs appear to maintain their central neural connections during the early stages of this degenerative process, the creation of conditions for the regrowth of RGC could allow the establishment of novel connections within the inner plexiform layer and the recovery of visual sensitivity. There is compelling evidence that the perineuronal environment within the retinal ganglion cell layer requires disruption as a first step to the promotion of dendritic plasticity and regrowth in the mature retina. I will discuss how that might be achieved using the rat glaucoma model as an example. In particular, I will address technical challenges that must be overcome if we are to provide convincing data that RGCs have recovered in experimental glaucoma.

## Course 15: Corneal Dystrophies – from molecular basis to therapeutic approach

### • 3631

#### Introduction, Surgical treatment options for corneal dystrophies

WYLEGALA E

Katowice

Introduction, Surgical treatment options for corneal dystrophies  
Corneal dystrophies are the group of hereditary disorders affecting all corneal layers. Since 2008 when IC3D classification was published there are new advances in diagnosis and treatment of corneal dystrophies. During this course we would like to present the current methods of diagnosis including genetic testing and different treatment options. The choice of the surgical treatment option depends on the corneal dystrophy type and includes: anterior lamellar keratoplasty for dystrophies without Descemet membrane involvement, descemet stripping endothelial keratoplasty for Fuchs corneal dystrophy and penetrating keratoplasty for dystrophies characterized by full corneal thickness involvement. All types of keratoplasty could be performed traditionally or with the assistance of the femtosecond laser. The corneal graft survival depends on the corneal dystrophy type and possible complication of the surgery related to the diagnosis of the corneal dystrophy is the recurrence of the disease.

### • 3632

#### Genetics of the stromal corneal dystrophies

NOWINSKA A

DRH Ophthalmology Department, Katowice

Genes connected to the occurrence of the stromal corneal dystrophies include: TGFBI (Transforming growth factor, beta-induced), CHST6 (Carbohydrate sulfotransferase 6), UBIAD1 (UbiA prenyltransferase domain containing 1), DCN (Decorin) and PIP5K3 (Phosphatidylinositol 5-Kinase type III). The description of each stromal corneal dystrophy genotype and phenotype will be presented based on the known literature as well as on the examples of eyes of the patients originating from polish population.

### • 3633

#### Classification of corneal dystrophies (IC3D)

NIELSEN K

Department of Ophthalmology, Arhus University Hospital, Aarhus C

Corneal dystrophies have been described and assigned various names in different regions at different times in history. A group of skilled cornea experts from four continents, the International Committee for Classification of Corneal Dystrophies (IC3D), reviewed the literature, agreed on common signs and symptoms, and presented a new classification system. As more corneal dystrophies become genetically characterized, this element has also been included in the system. The structure of the IC3D classification system will be discussed.

### • 3634

#### Imaging methods of the corneal dystrophies

JANISZEWSKA D

DRH Ophthalmology Department, Katowice

Corneal imaging techniques used for corneal dystrophies diagnosis and follow up include: optical coherence tomography, endothelial and confocal microscopy. In this part of the course we would like to describe different optical coherence tomography modalities and compare time and spectral domain OCT in visualization of the corneal deposits accumulated in the corneal dystrophies. We also would like to present the analysis of corneal morphology *in vivo* by confocal microscopy as well as describe the characteristic features of each corneal dystrophy in the histopathologic examination.

**• 3635****Genetics of the posterior corneal dystrophies**

DOBROWOLSKI D  
Katowice

**ABSTRACT NOT PROVIDED****• 3636****Meesmann dystrophy in Denmark**

NIELSEN K  
*Department of Ophthalmology, Aarhus University Hospital, Aarhus C*

Meesmann dystrophy is a rare corneal disease characterized by numerous epithelial microcysts. Three distinct families have been identified in Denmark (population 5.600.000 citizens). Clinical, histological and genetic similarities and differences will be discussed.

## SIS: FNAB for the diagnosis and management of intraocular tumours

### • 3641

#### Diagnosis FNAB for orbital and intraocular tumours from a cytologist point of view

KLIJANIENKO J

#### ABSTRACT NOT PROVIDED

### • 3642

#### Long term result of transcleral FNAB for genetic analysis of uveal melanoma

MIDENA E (1, 2)

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(2) GB Bietti Foundation, IRCCS, Rome

**Aim:** The aim of this study is to evaluate long-term safety and efficacy of in-vivo transcleral Fine Needle Aspiration Biopsy (FNAB) for cytogenetic testing of posterior uveal melanoma (UM).**Methods:** One hundred thirty-nine consecutive patients affected by UM underwent in-vivo 25-G transcleral FNAB just before brachitherapy. Sampled material underwent both FISH (chromosome 3 and 6) and MLPA analysis using standard procedures. Follow-up was longer than 36 months.**Results:** The use of 25-G transcleral FNAB appears a long-term safe and effective procedure for in-vivo cytogenetic testing of posterior uveal melanoma. Combined analysis of both arms of uveal melanoma bifurcated pathway (-3 and +6p) increase predictive value of FISH technique. MLPA allows obtaining more information than standard FISH in UM prognostication (partial chromosomes deletion).**Conclusions:** The biological and prognostic value of partial chromosome 3 deletion, as well as others subtle chromosomes alterations or complex MLPA results, remains unclear.

### • 3643

#### Curie experience with transvitreal and transcleral FNAB

CASSOLUX N  
Paris

Fine needle aspiration biopsies (FNAB) with different techniques have been recently developed during the management of intraocular tumors especially uveal melanoma. The need of a biopsy to diagnosed intraocular tumors remains rare. However in some difficult cases of achromatic tumors FNAB can be helpful to distinguish achromatic melanoma, metastasis, or choroidal malt lymphoma. FNAB is mainly used for choroidal melanoma prognostication. It has been shown that choroidal melanoma prognosis depends on well-known clinical factor but also genetic factors within the tumor (monosomy 3 and 8q addition or GEP class II). Determination of patient prognosis by FNAB open the way to adjuvant chemotherapy for high risk patients. Different techniques can be used to obtain sufficient tumor samples and these different techniques will be exposed during this symposium. Curie experience using transcleral or transvitreal FNAB will be described.

### • 3644

#### Incisional trans-scleral biopsy of choroidal melanoma

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Ocular Oncology Service, Liverpool

**BACKGROUND:** In our experience, trans-scleral fine needle aspiration biopsy (FNAB) does not provide sufficient tissue for all the required histological and genetic investigations, especially with thin tumours. **TECHNIQUE:** The author has developed a simple technique for performing incisional choroidal tumour biopsy, immediately before ruthenium plaque insertion. Briefly, the tumour is localized by transillumination and its margins are marked on the sclera with a pen. A plastic plaque template is temporarily sutured to the sclera with releasable bows and its position checked by trans-scleral transillumination and binocular indirect ophthalmoscopy. After removing the template, a rectangular, lamellar scleral flap is made, which is hinged posteriorly. A small incision is made in the deep sclera and several tumour samples are obtained with Essen forceps. The flap is closed with tissue glue, without suturing. The ruthenium plaque is then sutured to the sclera, using the pre-placed sutures. **DISCUSSION:** Tumor samples yielded using this method are larger than obtained with FNAB. The glue prevents tumour seeding into orbit and the lamellar flap protects the delicate ruthenium plaque surface from damage by the hardened glue.

**• 3645****How to handle ophthalmic biopsy specimen to optimize the results***COUPLAND S**Pathology, Dept. of Molecular and Clinical Cancer Medicine, Liverpool*

Ophthalmic biopsies are performed for diagnostic & increasingly for prognostic purposes. The aim of the presentation is to provide "tips and tricks" for the surgeon, ophthalmic technician and pathologist in the work-up of the typical and the "atypical" ophthalmic biopsy. The Liverpool Ocular Oncology Centre is one of three referral centres for adult ocular oncology in the UK. It receives close to 800 new referrals each year, with some of these being quite unusual cases. Contrary to practice in some centres, where there is a suspicion of choroidal metastasis of an underlying (possibly unknown) systemic tumour, an intraocular biopsy is performed as one of the initial investigations to confirm (or refute) the clinical impression. Morphological and immunocytological analysis can confirm the diagnosis and provide an indication of the site of the primary tumour. Further genetic analysis of the tumour cells (e.g. EGF-R mutations) may enable treatment choice. In the case of clear clinical diagnoses, biopsies are performed for prognostication purposes: e.g. in uveal melanoma. Close collaboration is required between surgeon and the pathology laboratory, in order to obtain the best diagnostic and prognostic yields from these samples.

• 3651

**Minocycline as a new neuroprotective agent in a rodent model of NAION**

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(2) Ophthalmology Hopital Pitié Salpêtrière, Paris

(3) Neuro-Ophthalmology, Wilmer Eye Institute Johns Hopkins School of Medicine, Baltimore

**Purpose** To study the neuroprotective properties of minocycline in a rodent model of NAION.

**Methods** 6 weeks old Long Evans male rats had induction of NAION in the right eye after intravenous injection of rose bengale and laser-induced photothrombosis of the head of optic nerve. Treated animals were injected intraperitoneally with minocycline 22mg/kg/day, 3 days before induction and until sacrifice. Controls were treated by PBS during the same period of time. SLO, fluorescein angiography, and OCT were performed at day 1, 8, 15 and 30 to monitor fiber loss. At day 30, all animals were sacrificed and flatmounted retina were stained with Brn3a. Retinal ganglion cells (RGC) were counted by manual method per field on stereology.

**Results** Sixteen rats had induction of NAION in the right eye, while the left eye was used as an internal control. In the PBS-treated group, fiber loss was observed on SLO from the day 8 post-induction. In this group, RGC loss in the NAION eye was 47.9% compared to control eyes ( $p < 0.05$ ). In the minocycline-treated group, RGC loss could not be observed on SLO, and stereology demonstrated a 9.58% loss compared to control eyes (not statistically significant).

**Conclusion** Minocycline seems to be an effective neuro protective agent in a rodent model of NAION, when administered before and after induction of NAION. The mechanisms involved in this neuroprotective effect are still to be further evaluated. This molecule could thus be a promising candidate, both in the treatment of NAION but also in the prevention of involvement of the fellow eye.

• 3652

**Retinal ganglion cell impairment in Leber Optic Neuropathy carriers triggers cortical compensatory plasticity in extrastriate cortex**

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(2) IBILI, - Institute for Biomedical Imaging and Life Sciences, Coimbra

**Purpose** To establish a link between silent retinal progressive impairment and cortical reorganization in a cohort of 15 asymptomatic patients harboring the 11778G>A mutation with good visual acuity and normal ocular examination (pre-clinical phase). We aimed to phenotype preclinical silent degeneration from the psychophysical, neurophysiological and structural point of view. Moreover we aimed to establish whether retinal measures could explain cortical reorganization.

**Methods** We studied RGC function at the population level using pattern electrophysiology and chromatic contrast sensitivity along three chromatic axes. We used optical coherence tomography to measure macular, RGC nerve fiber layer as well as inner and outer retinal layer thickness. We then asked whether such measures could explain previously identified cortical reorganization as assessed by cortical magnetic resonance imaging thickness measures in extrastriate visual cortex.

**Results** We found that compensatory cortical plasticity occurring in V2 and V3 is predicted by thickness of macular RGC axonal layer. This was also the most discriminative measure between carriers and controls, as revealed by ROC analysis. Moreover we found that the substantial cortical reorganization that occurs in the carrier state, can be used to provide statistical discrimination between carrier and normal groups to a level that is similar to measures of retinal dysfunction.

**Conclusion** We conclude that cortical compensatory plasticity in visual areas V2 and V3 is triggered by pathology in retinal ganglion cell axons.

• 3653

**incidence of Diabetic Macular Edema in type 1 Diabetes**

LUIJAIN I (1, 2)

(1) Ophthalmology, Jeddah

(2) Retina, Jeddah

**Purpose** Diabetic retinopathy is a serious vision threatening complication which can be prevented if detected early. Since diabetic retinopathy has been reported to occur only rarely before the end of pubertal development, children and adolescents are seldom included in screening programmes. Our main goal is to screen for early retinal changes and review the risk factors involved in the development of diabetic retinopathy in children.

**Methods** We prospectively enrolled 200 children and adolescents with insulin-dependent type 1 diabetes mellitus diagnosed before the age of 15.0 years (disease duration of < 12 years) and who were older than 5.0 years at the time of examination from our pediatric endocrinology clinics at 2 tertiary care hospitals (King Abdulaziz University Hospital and King Faisal Specialist Hospital) in Jeddah, Saudi Arabia. All patients received full eye examination including Best Corrected Visual Acuity. Retinal examination was performed with stereoscopic fundus examination by indirect ophthalmoscope for peripheral retinal changes and slit lamp exam with 75 D lens for macular edema. Ocular coherence tomography (OCT) was performed for all patients to evaluate sub-clinical macular edema.

**Results** Non of our patients had proliferative diabetic retinopathy changes, we also did not see evidence of diabetic macular edema. Mean diabetes duration was 3 years and mean HbA1c was 6.5%. Mild non proliferative DR was diagnosed in 30 children (15 %), those with DR were significantly older ( $P < 10-3$ ), had a longer duration of diabetes ( $P = 0.001$ ), and had higher HbA1c ( $P = 0.15$ ). After adjustment for age, only longer duration remained significantly associated with DR ( $P = 0.01$ ).

**Conclusion** Mainstay of prevention is tight glycemic control with regular screening and fundus examination, with OCT if suspension.

• 3654

**Telemedicine screening for diagnosis of retinopathy of prematurity in clinical practice**

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(2) CH, Le Havre

**Purpose** To compare the Retcam Shuttle used in telemedicine and performed by a non-ophthalmologist with the "gold standard" of binocular indirect ophthalmoscopy performed by a referent ophthalmologist for the diagnostic of retinopathy of prematurity.

**Methods** Retrospective analysis of the archival data gathered in 18 months between 1 May 2011 and 1 November 2012 in two centers: one practicing telemedicine and one using indirect ophthalmoscopy. The same ophthalmologist was reading the images obtained by Retcam in the first center and performing the fundus examination in the other center. A total of 255 infants were examined, 74 by Retcam and 181 by indirect ophthalmoscopy, resulting in 570 examinations (141 and 429 respectively).

**Results** The mean birth weight of the infants was 1300 grammes (SD 340 grammes) in the center practicing telemedicine and 1130 grammes (SD 440 grammes) in the center using indirect ophthalmoscopy. The mean gestational age were 28 weeks (SD 12 days) and 27 weeks (SD 19 days) respectively. A total of 1.8 examinations per infant were performed in the center practicing telemedicine, 2.4 in the other one. Retinopathy of prematurity was diagnosed in 13% of infants of the center using telemedicine and 20% of the other one.

**Conclusion** Telemedicine can be useful to diagnose retinopathy of prematurity especially in remote areas but it seems to have a lack of sensitivity. A very tiny collaboration between the center practicing telemedicine and the ophthalmologist should be sought to improve the results of screening.

• 3655 / F062

**Refractive error after intravitreal bevacizumab for threshold disease in retinopathy of prematurity: Two-years follow-up**

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**Purpose** To examine the association between myopic refractive error at two years of life in infants who postnatally received an intravitreal bevacizumab injection or standard laser therapy for threshold retinopathy of prematurity (ROP) in fundus zone I or zone II.

**Methods** In the retrospective non-randomized interventional comparative study, infants who consecutively received a single intravitreal bevacizumab (0.375 mg) injection (study group) were compared with infants who had previously undergone standardized retinal argon laser therapy (control group). The follow-up examination included cycloplegic refraction.

**Results** The study group consisted of 7 children (13 eyes) and the control group included 14 children (27 eyes). Both groups did not differ significantly in gestational age and follow-up. At the end of follow-up of 24 months, refractive error (median:+1.00 diopters (D) (+4.0D to -10.00D) versus median:-7.50D (+2.50D to -15.0D); $P=0.001$ ), astigmatism (median:0.25D (0D to 2D) versus median:1.25 D (0D to 5.75D); $P=0.001$ ) and prevalence of high myopia ( $\leq-6$ D) (15±10% versus 52±10%; $P=0.04$ ) were significantly less in the study group than control group. After adjusting for gestational age, follow-up time, gender and birth weight, refractive error ( $P=0.007$ ), astigmatism ( $P=0.01$ ) and prevalence of high myopia ( $P=0.03$ ; Odds Ratio:0.13) were significantly associated only with laser therapy versus bevacizumab therapy.

**Conclusion** A single intravitreal bevacizumab injection as compared to conventional retinal laser coagulation was associated with a lower degree of myopization and less astigmatism at two year follow-up.

• 3656 / F063

**Wide field imaging in patients treated with vigabatrin**

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(2) *Cabinet d'Ophthalmologie, Paris*  
(3) *Pédiatrie, Reims*

**Purpose** Vigabatrin is an active treatment for epilepsy in children particularly for West's syndrome. In children when visual field testing cannot be performed, regular ERG recording is mandatory to screen for retinal toxicity. Some peripheral retinal changes have been described with ophthalmoscopy. The purpose of this study was to evaluate the occurrence of retinal changes wide field imaging and to compare the results with ERG recordings.

**Methods** Five consecutive patients treated with vigabatrin were followed up with Flicker ERG and wide field imaging using scanning laser ophthalmoscopy (Optomap, Optos). Flicker ERG amplitude was considered as abnormal below 50 $\mu$ V.

**Results** Two patients had abnormal ERG recordings and presented with peripheral pigmentary changes. One patient presented with abnormal flicker ERG and normal retinal imaging. Two patients had normal ERG recordings with no peripheral changes on wide field imaging.

**Conclusion** The follow-up with ERG in children treated with vigabatrin is complicated because the recordings are regularly repeated. A follow-up protocol combining electrophysiological recordings and wide field imaging could simplify screening for retinal toxicity but the remains to be demonstrated with a prospective study.

• 3657 / F064

**Utility of systematic ophthalmological screening in congenital toxoplasmosis: Epidemiological study of a French cohort between 1990 and 2011**

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(2) *Parasitology, Strasbourg*  
(3) *Pediatrics, Strasbourg*  
(4) *Ophthalmology, Colmar*

**Purpose** Ophthalmologic complications of congenital toxoplasmosis, such as retinochoroiditis, are particularly feared. Any child with confirmed congenital toxoplasmosis is treated and regularly followed with many fundus examinations. The aim of our study is to describe the management and monitoring of a cohort of patients with congenital toxoplasmosis in Alsace, and the impact of this disease in terms of parental anxiety using a standardized questionnaire.

**Methods** Our study recorded 35 children with congenital toxoplasmosis, born between 1990 and 2011 in Alsace. All patients were followed by an ophthalmologist. A standardized questionnaire concerning the experience of pregnancy and post-natal follow up were submitted to parents.

**Results** At birth, a retinochoroiditis was detected in 2 children, and only one child developed the disease during routine monitoring. Brain abnormalities were noted in 3 children at birth, none of them presented with retinochoroiditis to this day. An average score of 15 out of 23 was found by our standardized questionnaire, reflecting significant anxiety due to congenital toxoplasmosis.

**Conclusion** Parental anxiety due to congenital toxoplasmosis is obvious. A directed follow-up by a complete pediatric examination at birth, including eye fundus, and good information on functional signs of ocular toxoplasmosis may improve the screening without impact on visual function.

• 3658 / F065

**Quality of life among children with severe and chronic ophthalmic conditions**

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**Purpose** To evaluate the impact on the quality of life of children from 8 to 18 years-old of a rare and serious ophthalmological condition (cataract and congenital glaucoma).

**Methods** Analysis of the answers obtained between May and November 2012 by autoquestionnaire (KIDSCREEN-10) among children from 8 to 18 years-old and from their parents and approaching their physical, social, moral and educational wellness. The children are patients of a department of pediatric ophthalmology of the South of France and followed-up on the long term for chronic and severe ophthalmological conditions.

**Results** 50 children (mean age 12 years) and 45 parents participated in this study. We register a lack of energy and an impression to be decreased to the everyday life to 32% of the children according to their parents (n: 14) and 27% (n: 13) according to the children. A feeling of sadness is present according to the parents for 43% of the staff (n: 19) and to 36% of the children according to their statements (n: 18). The solitude is felt at 64% (n: 28) of children according to their parents against 30% (n: 15) according to the children. The school life is affected according to the parents for 82% of the children (n: 37) and 70% when we question the children (n: 35). The global appreciation of the child's health is degraded at 30% of the children (n: 13) according to their parents against 22% according to the children (n: 11).

**Conclusion** Chronic ophthalmological conditions have certain noxious impact on the quality of life of the children and particularly in their social relationships and their school life. This study shows a good correlation between the felt of the parents on the real-life experience of their children and the appreciation which make the children themselves.

## SIS: Functional indicators for cataract surgery

### • 3661

#### Experience with straylight in the cataract clinic

*COCHENER B  
Brest*

We know that there are many sources of physiologic scatters such modified cornea (after refractive surgery, haze, dystrophies, ocular surface disease) , vitreous alterations and lens changes with the aging process inducing progressive cataract and impairing light diffusion. Nowadays, thanks to the access to scatter measurement, we might be able to assess tear film quality dynamics (with an increase in ocular surface desiccation commonly observed in old patients), but moreover we could detect early cataract at the stage of no induced visual acuity decrease but quality of vision degradation. We will present the preliminary results of a multicenter French study based on the use of the Double Pass Image Analyser (OQAS) for the potential diagnosis of cataract, on a standardized questionnaire of life and taking also account the integrity of ocular surface in addition to visual performances. The key parameter provided by this platform is the OSI (Ocular Surface that increases when scatters are in elevation (superior to 4). Our preliminary pilot results show a significant correlation between quality of life and OSI changes, suggesting that the objective measurement of light scatter might be of interest for detection of early cataract and that the consideration of quality of life (based on a basic questionnaire) should be considered in the modern new definition of cataract.

### • 3662

#### Objectivity and reliability of the C-Quant in a clinical setting

*GICQUEL JJ  
Poitiers*

The fact that the (C-quant) Straylight Meter measures the straylight as the subject actually sees it (and is disturbed by it) may be considered as a limitation of the technique, when used in a clinical environment. Because it is a psychophysical technique, reliability of individual measurements needs to be checked (as with visual field measurements). We decided to evaluate the objectivity and reliability in patients with cataract in a regular clinical environment. For this very purpose, we studied the reliability parameter (ESD), designed to predict the accuracy of an individual measurement. We also tried to simulate frauds.

### • 3663

#### Multiparameter cataract indication approach

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In the clinic, the indication for cataract surgery is often based on a visual impairment noted by the patient, the presence of cataract that accounts for the visual symptoms, and the likelihood of improved vision with surgery. The indication for cataract surgery is thus often based on subjective observations by the patient and the ophthalmologist. Visual acuity, especially when obtained through a pinhole, and straylight assessment may be more objective observations in the assessment for cataract surgery. This presentation will focus on the integration of these various subjective and objective parameters in the indication for cataract surgery.

### • 3664

#### Inclusion effects if straylight is added to the indication for cataract surgery

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In several European countries, criteria for cataract surgery (CE) are being debated. Within the ophthalmological profession, but also from other sides it is sometimes thought that more transparent choices are in order. Health insurances and governments may exert pressure for other than quality of care motives. With the high levels of safety nowadays achieved, CE can be offered much earlier, but to which patients precisely? Whatever the motives, it is of relevance to discuss potential improvements in targeting CE, making use of new instruments for evaluation of cataract related losses of visual function. As studies by Pesudovs and others show questionnaire assessment to be unreliable on individual basis, the focus must be on functional assessment. This presentation will focus on the CE inclusion effects if straylight assessment (log(s)) would be combined with visual acuity (logMAR). As one dimensional model for disease severity logMAR+log(s) (JCRS2012;38:840-848) is applied to population studies (5000 general population eyes AJO2007;144:358-363. 420 CE eyes JCRS2012;38:840-848. 160 refractive lens exchange eyes, Lapid et al. paper in preparation). In all studies straylight would add strongly to the number of CE inclusions.

*Commercial interest*

**• 3665****Cataract morphology, visual function and quality of life:  
A prospective observational study of surgical section**

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We sought to identify the relative importance placed on quality of life indicators amongst other patient-specific determinants of cataract surgery in a 12-month prospective study carried out at Princess Alexandra Eye hospital in Edinburgh attending 6 consultant teams. Data collection was carried out covering three major domains:visual function: (1) visual acuity, contrast sensitivity, retinal straylight measurement, (2) cataract morphology: Oxford cataract classification scale (3)quality of life:VF14, Massof and Sparrow Questionnaire. Patient factors predicting surgical selection reflective of current practice were best-corrected visual acuity, brunescence grades and straylight measures, rather than quality of life scores across all grades of presenting severity for all consultant teams. Reducing thresholds for surgery and a "ceiling effect" may explain the poor predictive power of vision-specific quality of life scales in current cataract surgical selection.

• 3671

**A unilateral chronic conjunctivitis as onset of a systemic sarcoidosis**

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**Purpose** Introduction: Granulomatous conjunctivitis is unusual as a presenting symptom in patients suffering from Sarcoidosis. We report the case of a 48-year-old patient, presenting a chronic unilateral conjunctivitis and dyspnea for two years. The conjunctival biopsy led to the diagnosis of systemic sarcoidosis.

**Methods** Case Report : A 49-year-old woman, came for an asymmetric bilateral eye discomfort for almost 6 months treated for a mislabeled allergic conjunctivitis, with no effect. She reported an effort dyspnea for almost 2 years, with no other signs. Visual acuity was 20/20 P2. At slit lamp we found a low break up time at 4s with meibomian gland dysfunction and normal Shirmer test. A thickening of the lower conjunctival fornix at OR, with the presence of granuloma was noted, without pre-auricular lymph node. Anterior segment examination was completely normal as intra-ocular pressure and fundus.

**Results** A conjunctival biopsy was performed and pathologic examination showed the presence of epithelioid granuloma and giant cell without caseous necrosis. All culture including mycobacteria, were negative. A medical check-up confirmed systemic sarcoidosis. The patient was treated topically by steroids. Systemic steroids with methotrexate relay were established

**Conclusion** Conclusion: Sarcoidosis can be, life threatening, especially by a heart affection that must be systematically tested. This clinical case shows the interest of a rigorous etiological investigation when a patient present a chronic unilateral or bilateral granulomatous conjunctivitis. The diagnosis of the sarcoidosis must be evoked and must motivate a general check-up, especially if respiratory symptoms are associated. A biopsy may be performed to confirm the diagnosis.

• 3673

**Simple novel surgical device to facilitate preparation of endothelial grafts for DMEK**

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**Purpose** Descemet's membrane and endothelial keratoplasty (DMEK) is the only technique to achieve a posterior corneal transplant without any interface. It is however difficult to achieve, mainly during the preparation of the graft. We propose a simple novel surgical device to facilitate this preparation.

**Methods** We used 20 corneas for research in this study. The device is in the form of a circular blade with a diameter of 8.5 mm. The blade is broken in two opposite points on 4 mm. The blade is applied to the cornea in order to cut Descemet's membrane and deep stroma. Preparation of the endothelial graft is performed on an artificial anterior chamber using a simple technique with a 27 gauge cannula to detach Descemet's membrane. The dissection is performed under the microscope by a corneal bank technician in 8 cases, by a resident in 4 cases by a cataract surgeon in 8 cases. The three operators followed a specific course on the same day of dissection but had never tried to dissect a DMEK graft before. Endothelial cell density (ECD) was measured before dissection and 3 days after storage in organ culture. Histological analysis was performed.

**Results** Descemet graft could be easily dissected in 19 cases and resident failed dissection in one case. ECD was 2534±275 cells/mm<sup>2</sup> before dissection and 2412±360 cells/mm<sup>2</sup>, after 3 days in organ culture ( $p<0.01$ ). Histological sections confirm that the cleavage was between DM and posterior stroma.

**Conclusion** We report here a reliable and efficient technique for the preparation of pure descemetic grafts.

**Commercial interest**

• 3672

**Lithium increases p63 levels in cultured human limbal epithelial stem cells**

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**Purpose** p63 (isoform ΔNp63α) is a putative stemness marker for limbal epithelial stem cells (LESCs), and is essential for the proliferative potential of stem cells in stratified epithelia. The niche for LESCs is the limbus, located between the clear cornea and bulbar conjunctiva. LESCs are responsible for and able to renew and repair the corneal epithelium throughout life. In a number of disorders and after trauma, limbal stem cell deficiency (LSCD) may occur, and this can be treated by isolation and cultivation of LESCs from a healthy eye followed by transplantation to the affected eye. It is known that the percentage of p63-bright cells affect the success rate of the transplantation. Thus, we wanted to reveal whether lithium, a known Wnt signaling inducing agent, may increase the expression of p63 in human LESC cultures.

**Methods** Small limbal biopsies (1x1-2mm) were dissected from donor corneas and cultured in a standard medium at 37°C and 5% CO<sub>2</sub> under different concentrations of lithium (LiCl<sub>2</sub>). The cells were cultured for 3-6 weeks and medium was changed every 2-3 days.

**Results** The epithelial cells migrated from the limbal biopsy to form an epithelial sheet. Lithium enhanced the expression of p63 compared to controls, and this was confirmed by qRT-PCR. Further studies mapping epigenetic alterations of the lithium treatment are currently conducted.

**Conclusion** Lithium induced upregulation of p63 in LESC could be clinically important by increasing the long term success of limbal stem cell therapy.

• 3674

**Allergic conjunctivitis related to cat and dog dander**

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**Purpose** A retrospective study on the prevalence of sensitization to cat and dog dander in patients with allergic conjunctivitis.

**Methods** 284 patients with allergic conjunctivitis (124 males-160 females), with age from 18 to 70 years old (mean age: 42.30 for males, 41.35 for females), underwent skin prick tests (SPTs) to cat and dog dander according to current guidelines.

**Results** The results showed that more frequent allergens (positive SPTs) were cat dander 67/284 (23.59%), while dog dander showed lower positivity rates 44/284 (15.49%). As regards gender, 33 from 67 patients with positivity to cat dander were males and 34 were females. The numbers for sensitivity to dog dander were 20 males and the rest 24 females, indicating no sex predilection.

**Conclusion** Symptoms and signs of ocular allergy are very common and may be related to sensitization to cat and dog dander among other factors. The patients with allergic conjunctivitis included in our study tended to have positivity to cat rather to dog dander. No sex predilection was observed.

• 3675

**Identification of infected corneal epithelial cells using an innovative in vivo fluorescent multilaser confocal microscope: Proofs of concept**

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**Purpose** To make the proof of concept of using fluorescent markers specific of certain bacteria or viruses (Herpes simplex virus, HSV) to make the aetiologic diagnosis of infectious keratitis directly at patient bedside, using fluorescent in vivo confocal microscopy (FIVCM)

**Methods** We fully adapted for ophthalmology, the dermatology multilaser FIVCM vivascope 1500ML (Lucid Inc, NY, MAVIG GmbH, Germany) equipped with 488, 658, and 785nm class IB lasers. Human corneas unsuitable for graft were infected with either a specific bacteria species or HSV suspensions after mechanical superficial central wound to mimick a superficial infectious keratitis. They were incubated with the suspension during 2h, rinsed, and returned to storage medium for 24h at 31°C. For controls, bacteria or viruses were replaced by balanced salt solution. Corneas were incubated with the corresponding fluorescent marker, one for HSV, the other for bacteria during 1h at 37°C. After meticulous rinsing, corneas were mounted on an anterior artificial chamber and observed with the FIVCM

**Results** Control corneas showed little to no fluorescence, whereas numerous epithelial cells were fluorescent using the corresponding couple marker/wavelength

**Conclusion** For the first time we obtained the POCs, on ex vivo human corneas, of specific diagnosis of bacteria or HSV keratitis using FIVCM. Animal experiments are ongoing in our laboratory on infectious keratitis models to confirm the feasibility and safety of this new promising diagnosis method. Grant: Appel à projet jeune chercheur GIRCI Rhone Alpes Auvergne 2012

• 3676 / S017

**Various epithelial manifestation of herpetic keratitis in corneal confocal microscopy imaging**

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**Purpose** To present different variants of epithelial changes observed in patients with herpetic keratitis in corneal confocal microscopy in vivo images.

**Methods** In 19 patients with diagnosed herpetic keratitis of typical clinical course, we performed the following: basic slit lamp examination, corneal scans using Scheimpflug camera and corneal confocal microscopy in vivo. Full thickness corneal scans were made and compared with corresponding images from healthy objects. Additional imaging of epithelial layer with higher magnification was made to compare detailed changes within all components of epithelial layer.

**Results** On a slit lamp examination we described different size and surface ulceration of typical dendritic shape. In the image from Scheimpflug camera – local turbidity of stroma and lack of superficial layers in place of ulceration. The corneal confocal microscopy images showed different severity of stromal changes in form of hazing, turbidity, keratocytes activation, inflammatory cells infiltration or fibrosis. In epithelial layer scans except mostly diffused inflammatory infiltration, we described changes involving: epithelial cells-intracellular inclusions, abnormal cells shape and size, degeneration; intercellular matrix-hazing, inclusions; superficial nerve plexus-thinning or lack of nerves, mace expand of nerves, nerve fibers proliferation; Bowman membrane and anterior stroma-epithelial hernias into deeper layers.

**Conclusion** Herpetic corneal inflammations have no typical image in corneal confocal microscopy; however can show repeatable features in epithelial layer, which are helpful in diagnosis. Corneal confocal microscopy is useful tool for recognizing viral keratitis.

## SIS: The myofibroblast: a key player of tissue repair

• 3711

### Myofibroblast biology and interactions with the environment

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Normal tissue repair includes number of overlapping phases. After injury, there is usually an early inflammatory step. Next, as granulation tissue forms, fibroblasts invade the wound and begin to replace the provisional matrix with a more mature wound matrix. As the granulation tissue phase proceeds, fibroblasts acquire a new phenotype with prominent microfilament bundles. These typical myofibroblasts (MFs) have been shown to develop a smooth muscle-like phenotype, and are responsible for wound contraction. Lastly, in the resolution phase of healing, there is considerable loss of cell types including MFs, by apoptosis. Inappropriate delay of apoptosis, and thus increased survival of MFs activated during the healing process, may be a factor which leads to pathological situations and excessive scarring. In most situations, MFs derive from local connective tissue cells but it has now become clear that other cells can contribute to MF production. Both MF differentiation and apoptosis are dependent on cytokines, mechanical stress and more generally on cell-cell and/or cell-matrix interactions. The control of the MF activity represents an important target to promote healing and to limit excessive scarring.

• 3712

### Fibrotic alterations in glaucoma

WALLACE D

Glaucoma is the second leading cause of irreversible blindness worldwide, thought to affect 60 million people. It is a chronic progressive optic neuropathy with characteristic changes in the optic nerve head and subsequent visual field defects. In glaucoma, the lamina cribrosa (LC) region undergoes thickening and posterior migration in the early stages of the disease process, and later undergoes shearing and collapse of the LC plates leading finally to a thin fibrotic connective tissue structure/scar. This is characterised by the build-up of extracellular matrix material and it is thought that this accumulation eventually results in elevated intraocular pressure and subsequent nerve damage. Here, we directly address the ongoing fibrotic pathology by use of alpha-smooth actin expressing (myofibroblast-like) human primary LC cells, including mechanisms of fibrosis and potential anti-fibrotic therapies. Additionally we discuss the potential role of the Retinal Pigment Epithelial cell as a mediator of glaucomatous optic disc cupping / fibrosis through epithelial to mesenchymal transition.

• 3713

### Regeneration of lymphatic vessels after lymphatic-specific photodynamic therapy is not dependent on lymphangiogenesis and tissue remodeling by myofibroblast

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Photodynamic therapy (PDT) is a clinically used therapeutic procedure based on the administration of a non-toxic photosensitizer followed by sub-thermal light exposure for treatment of pathologic cornea neovascularization. However, this approach does not target pre-existing lymphatics at the cornea limbus and sustained antigen drainage can lead to rejection of allogenic cornea grafts. Our PDT protocol aim at specific destruction of lymphatic vessels leaving blood circulation intact. Collecting lymphatic vessels remain occluded for 9 days and then the drainage is restored. Interestingly, lymphatic regeneration occurs not by lymphangiogenesis and wound healing but by true, epimorphic regeneration. We found that myofibroblasts were present in the tissue even though the tissue injury was limited to the lymphatic endothelial cells of collecting lymphatics and their pericyte coverage. Even though myofibroblasts were recruited and persisted in the tissue for the whole period of lymphatic regeneration no scarring in other tissue structures was observed, i.e. remodeling of blood vessels, nerve fibers and adipocytes. Limited tissue remodeling and scarification after lymphatic-specific PDT should alleviate potential clinical translation of this method for temporal occlusion of collecting lymphatics e.g. before cornea grafting.

• 3714

### Role of myofibroblasts in epiretinal membrane development

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Purpose: Fibrous epiretinal membranes (ERMs) result from inappropriate proliferation, migration and differentiation of several cell types including myofibroblasts. They occur at the endstage of proliferative and idiopathic vitreoretinopathy, and proliferative diabetic retinopathy. Myofibroblasts exhibit contractile features that are typical of granulation tissue during wound healing and fibrocontractive diseases and are responsible for granulation tissue remodeling and retraction. The marker of myofibroblasts is the expression of alpha-smooth muscle actin (alpha-SMA). Transforming growth factor-beta1 (TGF-beta1) and ED-A fibronectin are key players of the complex process of myofibroblast differentiation. Methods: Samples of ERM were studied by electron microscopy, immunohistochemistry, and confocal microscopy with antibodies specific for alpha-SMA, vimentin, TGF-beta1, TGF-beta receptor II and ED-A fibronectin. Results: The presence of alpha-SM actin-positive myofibroblasts was associated with the expression of vimentin, TGF-beta1, TGF-beta receptor II, and ED-A fibronectin in all types of ERMs. Conclusions: The results furnish new data on the mechanism of alpha-SM actin stimulation in fibroblasts in a human pathologic setting.

• 3721

### Glaucoma surgery

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**Purpose** Discussion of surgical management of different types of glaucoma.

**Methods** In congenital glaucoma early surgical management is indicated (goniotomy). In acute angle-closure glaucoma immediate treatment is imperative (laser iridotomy or surgical iridectomy). In chronic angle-closure glaucoma management depends on the evolution and duration of the process:a) Peripheral iridectomy.b) Non-perforating trabeculectomy.c) Perforating trabeculectomy.d) YAG laser cyclocoagulation. In chronic simple glaucoma medical therapy is indicated if permanent (24 hours) response of the pressure is obtained. If not lamellar trabeculectomy with peripheral iridectomy and application of mitomycin is attempted. In severe chronic simple glaucoma perforating trabeculectomy with mitomycin is indicated.

**Results** In case of flat anterior chamber, with hypertension, malignant angle closure glaucoma is suspected. Management: a) Posterior sclerotomy. b) Vitreous aspiration. c) Reformation of the anterior chamber with air and Healon. d) Application of atropine 4%. If an incipient cataract is present simultaneous sonophako with IOL implantation is indicated. In case of flat anterior chamber with ocular hypotension instillation of 4% atropine and posterior sclerotomy for evacuation of the choroidal detachment, followed by restoration of the anterior chamber with air, is indicated.

**Conclusion** The main symptom of glaucoma is persistent increase of intraocular pressure which generally requires surgical intervention. The individual management has to be carefully selected according to the different types of the disease.

• 3722

### Reducing high intraocular pressure with hypertonic saline before eye surgery

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**Purpose** To compare the effect of intravenous hypertonic saline (IVHTS) on intraocular pressure (IOP) among patients with exfoliation glaucoma (ExG), primary open-angle glaucoma (POAG), and ocular hypertension (OHT).

**Methods** A prospective, interventional trial. Included were patients with an IOP 24-30 mmHg with or without topical medication. Excluded were patients using oral acetazolamide medication, those with heart or kidney failure, dementia or other systemic condition that markedly decreased physical performance. Participants received a bolus of 23.4% IVHTS through an antecubital vein. Infusion rate was 1 ml/s and dosage 1.0 mmol/kg sodium in all patients. We measured IOP before we injected the bolus (baseline), every minute after injection for 10 minutes, and then less frequently for 2 hours.

**Results** A total of 35 patients participated (mean age 69 y, standard deviation [SD] 9); 16 ExG, 13 POAG, and 6 OHT patients. The baseline IOP 26.2 (SD, 1.7) mmHg was significantly reduced two minutes after treatment to 22.4 (SD, 2.6) mmHg ( $P<0.001$ ). Maximum IOP reduction was achieved after 10 minutes, at which time IOP was reduced to 17.3 (SD, 2.9) mmHg ( $P<0.001$ ), and the mean percentage IOP reduction was 34% (SD, 9). Mean IOP reduction was similar between groups ( $P=0.351$ ; One-Way ANOVA). Two hours after treatment, the mean percentage IOP reduction was 26% (SD, 14). Within 1-2 minutes of treatment, 31 patients felt some sensation (pain, pressure) in the infusion arm, and 22 patients reported a feeling of warmth in their head.

**Conclusion** Hypertonic saline seems to be an effective and a rapid method to reduce IOP. This reduction seems to be independent of topical medication or glaucoma subgroup. IVHTS could be a practical method to reduce IOP before or during eye surgery.

• 3723

### STARflo™ Glaucoma Implant: 12 month clinical results

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**Purpose** STARfloTM Glaucoma Implant is made entirely with silicone STAR® Biomaterial, a flexible tissue-friendly, micro-porous structure designed to reduce fibrotic response and maximize long-term performance. Here we share our 12months experience with the first patients implanted with the STARflo.

**Methods** Four patients with advanced open angle glaucoma underwent implantation. Under anesthesia, a conjunctival flap and a superficial scleral flap were created. An incision in the second layer of sclera until choroid was performed to insert the implant body in the suprachoroidal space while the STARflo head was inserted into the anterior chamber. The scleral flap was sutured tight.

**Results** Patient ages were 65.3 years. Mean pre-operative IOP was 37.0 mmHg and mean pre-operative glaucoma medication was 3.25 intake/day. At 12 months (3 cases), mean IOP was 14.3 mmHg and mean glaucoma medication was 1.5 intake/day. No adverse events were reported during or immediately after the surgical procedure and no device-related adverse events were reported during follow-up. Early post-operative complications included transient hypotony, transient choroidal hemorrhage and transient abnormal macula. Bleb disappeared between month 1 and month 3. One patient, with posttraumatic COAG, was dropped out following a cyclophotocoagulation.

**Conclusion** Clinical results for STARflo have met the safety and performance endpoints of the study and shown encouraging results in the control of the IOP with a reduction of glaucoma medications. Although long-term success still has to be demonstrated, this newly-CE marked device is promising as a novel, suprachoroidal implant for bleb-free, IOP reduction for patient suffering from refractory open angle glaucoma.

**Commercial interest**

• 3724

### Trabeculectomy revision surgery with Ologen biodegradable collagen matrix implant

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**Purpose** To investigate the safety and efficacy of Ologen (OLO) implant (Kestrel Medical Ltd, Broadstone, Dorset) for trabeculectomy revision surgery versus trabeculectomy revision with Mitomycin C 0.3mg/ml.

**Methods** This study was a prospective pilot trial with 6-months follow-up. Fourteen glaucoma patients underwent trabeculectomy revision with OLO. Primary outcome measures included postoperative target intraocular pressure (IOP), with or without medications. Secondary outcome measures included bleb evaluation, OCT imaging, number of medications, number of postoperative interventions and adverse events. All patients were reviewed postoperatively at day 1, 7, week 3, 7, 12 and 24.

**Results** The mean pre-operative IOP was 22.3 ( $\pm 4.1$  mmHg), mean postoperative IOP was 14 ( $\pm 5.8$  mmHg). IOP reduction from baseline was statistically significant ( $p<0.05$ ). The bleb remained well formed and functioning in all patients at all postoperative reviews. OCT imaging allowed monitoring of the position of OLO implant and showed a progressive decrease in thickness from week 1 to week 7. No serious events were observed.

**Conclusion** The trial showed that trabeculectomy revision with OLO implant was safe and achieved a success rate similar to trabeculectomy revision with MMC.

• 3725

**Efficacy and safety of combined trans-scleral cyclophotocoagulation (TSCPC) and phacoemulsification surgery**

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**Purpose** To assess the effect of combined TSCPC and phaco+IOL with respect to; a) IOP ; b) VA; c) topical therapy d) side effect profile at 6 months post surgery.

**Methods** A retrospective review of 24 eyes undergoing combined TSCPC and phacoemulsification was conducted at 6 months post-operatively. The procedures were performed by one surgeon (EA) under peribulbar local anaesthesia. TSCPC was applied across 270 degrees after completion of phaco+IOL. Subtenons Triamcinolone Acetonide 40mg was administered after each case. G. Prednisolone 1% x6 daily was used post-operatively for 6 weeks. The patients were reviewed at weeks 1, 4, 12 and 24.

**Results** 24 eyes were included. 20 had open angle glaucoma, 3 had mixed mechanism glaucoma and 1 neovascular glaucoma (NVG). The average total energy used was 87.5W, (Range: 33.8-346W). Mean pre-treatment IOP was 22.1mmHg (Range 12-47mmHg) and mean IOP at 6 months post treatment was 14mmHg (Range 0-20mmHg);(p<0.05). Mean total medication was reduced from 2.1 to 1.9;(p=0.55). Diamox was discontinued in all 7 cases who were taking this pre-operatively. VA was stable or improved in 18 eyes (76%) and worse in 6 eyes (25%). Uveitis occurred in 5 eyes following treatment, 1 patient developed optic atrophy and chronic hypotony occurred in only 1 eye which had NVG. The causes of worsening of visual acuity at 6 months were chronic hypotony (n=1), chronic cystoid macular oedema (n=2), worsening of glaucoma (n=3).

**Conclusion** Combined TSCPC and phacoemulsification is effective in treating cataract and glaucoma. Post-operative inflammation is a potential threat to visual improvement.

• 3726 / T050

**Argon laser trabeculoplasty (ALT) for advanced glaucoma: A 12 months follow-up study**

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**Purpose** ALT has been used for lowering the intraocular pressure (IOP) for over 25 years. The aim of this study was to investigate the safety and efficacy of ALT in cases of advanced glaucoma unsuitable for surgery due to ocular or systemic conditions.

**Methods** This retrospective observational study had the following inclusion criteria: advanced POAG with uncontrolled IOP, maximum medical treatment, previously failed ALT or filtration surgery and unsuitability for surgical intervention due to ocular or systemic comorbidities. The data collected included: pre- and postoperative number of medications, visual acuity (VA), visual fields mean deviation (VF MD), IOP and adverse events during the study period. All ALT treatments were carried out by the same clinician.

**Results** A total of 40 patients (40 eyes; mean age: 79 years; range 56-92 years) were identified. The number of medications remained unchanged in 75% patients, decreased by 2-4 medications in 12.5% of patients and increased by 1-2 in 12.5% of patients. Mean Log MAR VA (baseline 0.27±SD 0.45; final 0.19±SD 0.3) and the VF MD (baseline -9.17±SD 8.9; final -9.45±SD 9.3) did not change significantly over the 12 months study period (Log MAR p=0.29; VF MD p=0.18). The change in IOP was statistically significant, p=0.00018 (p<0.001), with an average IOP reduction by 18.14% at 12 months. In 76% of patients the mean IOP decreased by 6 mmHg and in 47.5% of patients the final IOP was lower than 16mmHg. No adverse events were observed during the study.

**Conclusion** ALT appeared safe and effective in lowering the IOP in cases of advanced glaucoma unsuitable for surgery.

• 3727 / T066

**Motion perception in early glaucoma**

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**Purpose** The aim was to underline changes in the motion perception for early glaucoma. Our hypothesis consisted in inquiring if the impairment of the magnocellular pathway may modify the motion perception.

**Methods** We included 14 healthy subjects and 14 patients with early primary open angle glaucoma. A moving target was presented on a semicircular screen; participants were asked to localize the Ending Point (EP) of each motion. A stimulus consisted in a white dot moving horizontally. Two different laws of motion were displayed: a "biological" motion, consisting in a bell-shaped velocity profile, and a "non-biological" motion corresponding to a constant velocity profile. EP may be displayed in the pericentral or peripheral visual field. The experiment was constituted by 192 trials. We calculated the PCE (Position Constant Error) which was defined as the average difference between the estimation of the EP indicated by the participant versus the true location of the target. The PVE (Position Variable Error) was defined as the standard deviation of the responses of each participant.

**Results** All the participants overestimated the EP (13.23±8.87 mm for healthy subjects and 17.06±13.07 mm for glaucoma patients, p=0.2518). The PVE was 28.14±6.86 mm for healthy subjects and 40.95±9.83 mm for glaucoma patients (p=0.0004). There was a significant difference in the PVE for both groups when stimulus moved according to the "non-biological" velocity profile (p=0.0001). Glaucoma patients had substantial PVE increase when the target image had a "non-biological" speed profile (p=0.0388).

**Conclusion** The unexpected lack of difference between normal subjects and patients in localizing a moving stimulus suggested that the visual deficiency could be partly compensated by endogenous informations.

**• 3731****The limbal stem cell niche***SHORTTAJ (1, 2, 3)*

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Stem cells contain intrinsic mechanisms that control their behaviour but the environment immediately surrounding them, known as the niche, also plays a fundamental role. In a host of tissues and organisms the stem cell niche has been shown to maintain stem cells in an undifferentiated state and to regulate their behaviour. This stem cell – niche principle is typified by the corneal limbus. When limbal epithelial stem cells (LESC) are dissociated from their niche in the limbal palisades of Vogt they rapidly differentiate and cease to function as stem cells. It might be imagined that an understanding of the LESC niche would be of the highest priority for researchers in this field but the existence of a surrogate niche, in the form of growth arrested 3T3 fibroblast feeder layers, has allowed research on LESC biology to progress in the absence of a thorough understanding of their native niche. Thankfully LESC niche research has been gathering momentum and several exciting developments hold promise for limbal stem cell deficient patients. Amongst these are the characterisation of the physical structure of the niche and the identification of mesenchymal niche cells, which themselves displaying stem cell properties.

**• 3732****Assessment of the limbal condition***GICQUEL JJ**Poitiers*

New sources of autologous tissue that can functionally replace the corneal epithelium; have recently been considered as an alternative to allogenous limbal transplants for limbal stem cells deficiency (LSCD). Nevertheless, regardless of the cell expansion technique (*in vivo* or *ex vivo*), the assessment of the limbal condition is crucial. It goes from the initial staging at the acute phase of a severe chemical burn; using the right classification, to the evaluation of the remaining ocular surface inflammation, during the chronic phase. We will review all the aspects of the evaluation of the limbal condition, that help the clinician, build his therapeutic strategy.

**• 3733****Transplantation of cultured limbal epithelial cells***BORDERIE V*  
*Paris***ABSTRACT NOT PROVIDED****• 3734****Transplantation of cultured oral mucosal epithelium***BURILLON C*  
*Lyon***ABSTRACT NOT PROVIDED**

## Course 16: Stem cells for treatment of eye diseases

### • 3735

#### Bioengineering and stem cells for corneal endothelial cell therapy

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The global planetary scarcity for corneal donation pushes research teams to develop substitution strategies. The worldwide success of endothelial keratoplasty (EK) has profoundly simplified the paradigm of corneal bioengineering since the technical and biological requirements for EK is far simpler than for full thickness corneas. Two main concepts have recently emerged: bioengineered endothelial cell (EC) sheets inserted into the anterior chamber or EC suspension ready to be injected. Review of the literature and personal works from our laboratory of corneal bioengineering. According to the local availability of donors, researchers either focus on the optimization of the use of human corneas or on the development of alternative substitutes comprising transformation of primarily non-ocular human cell types (hECS, hIPS or MSC) and bioartificial or animal decellularized substrates as a carrier. We chose to use only human tissues and developed different methods able to multiply the EK available. With femtosecond laser cut, 5 to 7 EK seemed feasible with 1 donor. We also patented a corneal bioreactor helpful during the bioengineering process itself as well as for preclinical validation of the new advanced therapeutic medicinal products on ex vivo human corneas. Numerous proofs of concepts have been obtained and several preclinical animals studies done. The very first-in-man applications was done in Japan with injection of cultured EC. All these promising complementary strategies may constitute the tomorrow endothelial dysfunction treatments. Grants: ABM 2013, ANSM 2012, EFS 2012

### • 3736

#### Pluripotent stem cells (ES & iPS) for treatment of retinal dystrophies

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The impaired or complete loss of function of photoreceptor cells or supporting retinal pigmented epithelium (RPE) is the main cause of irreversible blindness in retinal diseases, such as inherited retinal degenerations and age-related macular degeneration (AMD). Rescuing the degenerated retina is a major challenge and cell replacement is one of the most promising approaches with the use of pluripotent stem cells. Human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells that have the ability to be expanded indefinitely in culture whilst retaining their pluripotent status could be used as an unlimited source of retinal cells for tissue transplantation. The current challenge is actually to determine culture conditions for efficient and directed differentiation of human ES/ iPS cells into RPE or photoreceptors, so that large numbers of these cells might be transplanted at the optimal stage. We will present several approaches allowing the generation of RPE cells from human ES and iPS cells; and we will report the ability of these stem cells to be committed into the neural retinal lineage, and further to be differentiated into photoreceptor cells following different protocols.

## • 3741

**Animal models for ocular melanoma**

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Animal models play an important role in understanding tumor growth and studying novel therapies in human cancer research. The significance of results from animal experiments relies on the selection of the proper animal model. Many attempts have been made to create appropriate models for uveal melanoma and its characteristic metastatic route. Ocular models have used Greene melanoma, murine B16 melanoma and human uveal melanoma cells, among others, in hamsters, rats, rabbits and mice. Various effective inoculation techniques, including subcutaneous, anterior chamber and posterior compartment injections have been developed to obtain tumor growth and mimic the pathological process of uveal melanoma. Metastatic models by subcutaneous, intraperitoneal, intravenous, intrahepatic, intrasplenic and intracardiac injection of tumor cells are able to induce tumor spread and simulate the human metastatic behavior to some extent. However, when we choose animal models, we must be conscious that the disadvantages, such as more aggressive tumor growth, the need for immune suppression in xenogeneic grafts and unreliable spreading sites should be taken into account.

## • 3742

**Use of xenografts for preclinical drug testing**

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Uveal melanoma(UM) originates from melanocytes just like cutaneous melanoma (CM) and similar to CM, the MAPK pathway is involved in the development of UM. However, *in vitro* we showed an inverse correlation between MAPK and c-Met activation with increasing metastatic potential of UM. In this study, we aimed to target both Src and c-Met with respectively Dasatinib and Crizotinib in an experimental xenograft model. We used tissue derived from three different human primary tumours which were subcutaneously transplanted in mice. We used the c-Met inhibitor Crizotinib to inhibit c-Met function and Dasatinib to inhibit MAPK via Src. We monitored pharmacodynamics and analysed treatment efficacy with kinase assays. Growth inhibition was seen after treatment with Dasatinib in all three tumours. Crizotinib treatment had no marked effect on tumour size but showed, like Dasatinib, substantial effects on kinase activity. Src and c-Met show susceptibility towards their inhibitors *in vivo*. Dasatinib showed an effect on both tumour growth and MAPK activity where Crizotinib effects remained limited to c-Met inhibition. Combined, these results support our *in vitro* pathway analysis and warrants for clinical trials with these compounds in UM.

## • 3743

**Development of uveal melanoma xenografts: From patient tumours to patient derived xenografts characterization**

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50% of uveal melanoma patients die from their metastatic disease showing the necessity to develop efficient therapeutic agents and useful and relevant tool for their preclinical assessment. Thus, we have established and characterized an *in vivo* panel of xenografts directly obtained from patient uveal melanoma tumors. 90 Samples obtained from patient primary tumors or metastases were subcutaneously grafted into SCID mice. 25 patient derived xenografts (PDX) were obtained and 16 have been characterized. UM histology of the PDX was confirmed by pathological analyses. NA17, Tyrosinase, and Melan-A antigen expressions were positive in all samples. Bcl-2 protein was overexpressed in almost all PDX. GNAQ, GNA11 and BAP1 mutations were observed in 4, 10 and 7 of 15 xenografts. The comparison of genomic alterations showed the same DNA damages as the corresponding patients and the differential genes expression observed was mainly due to difference of stroma between patient tumor and PDX. *In vivo* therapeutic assessments were being performed and will be presented. Our panel of PDX presents the characteristics similar to the patient's originated tumors and could constitute a useful preclinical tool for testing new agents and protocols.

## • 3744

**Orthotopic xenografts in ocular tumours**

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In order to allow pharmacological assessment of innovative intraocular treatments in more relevant and predictive models, we have developed preclinical orthotopic xenografts of both primary human uveal melanoma (UM) and retinoblastoma (Rb) into immunodeficient mice. Orthotopic models of human UM and Rb have been developed from subcutaneous xenografts previously established and characterized in the laboratory. Mice bearing xenografts were sacrificed and tumors were dissected and injected into the subretinal space of SCID mice. After sub-retinal injection, ophthalmic examination of the mice was performed weekly. When tumor cells invaded vitreal cavity and anterior chamber, the mice were sacrificed for ophthalmological pathological analyses. The RB200 model, was used for preclinical trial validation. After orthotopic transplantation, pathological analyses confirmed the conservation of histological subtype of the primary tumor, i.e. epithelioid and/or spindle cell, than in corresponding patient's tumors and subcutaneous xenografts. For retinoblastoma, the orthotopic xenograft showed similar clinical and pathological similarities to human tumors. These models are suitable for preclinical trial.

**SIS: Cell migration and xenografting in ocular melanoma****• 3745****Stem cells in conjunctival melanoma xenografts**

KSANDER B

Boston

**ABSTRACT NOT PROVIDED****• 3746****miRNA and conjunctival melanoma migration**

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**Purpose :** In cutaneous melanoma, downregulation of miR-211 has been implicated in migration and invasion. As we have previously demonstrated the downregulation of miR-211 in conjunctival melanoma *in vivo*, we evaluated *in vitro* the role of miR-211 in the migration of 4 conjunctival melanoma cell lines compared to a cutaneous melanoma cell line.  
**Methods :** Migration assays were performed using a cutaneous melanoma cell line and 4 conjunctival melanoma cell lines. Taqman RT-PCR was performed to evaluate the level of miR-211 in each cell line.  
**Results :** Compared to the cutaneous melanoma cell line with a high migration potential, 2 conjunctival melanoma cell lines demonstrated moderate migration abilities while 2 others no migration. The migration potential of the cell lines was significantly inversely correlated with Taqman evaluation of miR-211 expression ( $p=0.0239$ ).  
**Conclusions :** The migration ability of conjunctival melanoma cell lines is lower than a cutaneous melanoma cell line. The level of miR-211 expression in conjunctival melanoma appears *in vitro* to inversely correlate with the migration potential of the cell lines, confirming the downregulation of miR-211 observed *in vivo* in conjunctival melanoma.

**• 3751****Molecular Imaging of Protein and RNA Biomarkers in Retinal Vascular Disease**

JAYAGOPAL A

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Approaches for imaging disease biomarkers in the retina are needed to improve early detection, monitoring of disease progression, and assessment of therapeutic response. We will discuss our recent efforts toward *in vivo* imaging of protein, messenger RNA (mRNA), and microRNA (miRNA) biomarkers in animal models of retinal vascular diseases, including diabetic retinopathy and choroidal neovascularization.

**• 3752****In Vivo Bioimaging of RPE cell death**

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We have now demonstrated the ability to non-invasively image RPE cell death in a targeted fashion with a custom engineered NIRF dye conjugated to an irreversibly binding pan-caspase substrate. The bio-probe detection was sensitive and specific for degenerating RPE and overlying outer retinal layers in a mouse model with geographic atrophy (GA)-like features. No significant ocular toxicity was noted, and eyes maintained a clear visual axis with undetectable anterior or posterior segment inflammation. While the potential role of caspase inhibition in the treatment of GA remains uncertain and will be addressed in future studies, an ideal translation of this knowledge would be to utilize caspase imaging as a means for the early detection, prognosis, and indication for therapeutic intervention in GA.

**• 3753****Retinal Oximetry – Metabolic Imaging of the Retina**

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Diagnostic imaging of retinal disease has been limited to structural analysis, angiography and testing of visual function. Until the advent of retinal oximetry, no metabolic retinal imaging has been available in the clinic. Metabolic imaging has obvious relevance to metabolic disease such as diabetic retinopathy and oxygen imaging is particularly appropriate for ischemic disease such as retinal vein and artery occlusion. Fundus camera based spectrophotometric retinal oximeters have allowed clinical studies over the last several years. Remarkably, abnormalities in retinal oxygen metabolism have been discovered in most major retinal diseases, including diabetic retinopathy, retinal vein occlusions, glaucoma and age related macular degeneration. These results open a new window into the pathophysiology of these diseases and may help evaluate severity and progression.

**• 3754****DARC, Sick and Dying Cells**

CORDEIRO M

*London*

DARC (Detection of apoptosing retinal cells) is currently being taken to clinical trial as a new diagnostic and screening imaging technique of retinal abnormalities. Initially it will involve patients with primary open angle glaucoma, where apoptosis of retinal ganglion cells (RGCs) is an early event. Early detection of RGC apoptosis and its prevention of this process holds promise as a novel and effective treatment for glaucoma, especially as recent evidence has suggested a reversible element.

## SIS: Oxidative stress and cataract

### • 3761

#### **Glutathione and Ascorbate: The balance between oxidant and carbonyl stress in the aging human lens**

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**Background:** Two major processes appear to be at play in formation of ARNC. On the one hand advanced glycation end products (AGEs) modify lens crystallins, increasing thereby their susceptibility to oxidation via unfolding, exposure of SH-groups and formation of UV-photosensitizers, on the other hand SH groups form disulfides and high molecular weight aggregates that scatter light. In order to find out if there are critical SH residues in the latter process, we have generated a glutathione knockout(LEGSKO) mouse and an in vitro model of crystallin aggregation upon oxidation with H<sub>2</sub>O<sub>2</sub>. **Methods:** Crystallins from in vitro incubation or LEGSKO mouse were analyzed using ICAT labeling methods and 2D gel electrophoresis (2D-PAGE) for disulfide analysis. Incubation of mouse lens homogenate (LH) with 5 mM H<sub>2</sub>O<sub>2</sub> led to opacification within a few hours. **Results:** Analysis of LEGSKO crystallins by 2D-PAGE revealed an age-related shift from mostly intramolecular to intermolecular disulfide crosslinks which mimicked the pattern observed at 20 months in wild type lenses. All oxidation-related disulfide forming sites in 13 crystallins were identified in vitro and found quasi concordant with those forming in the LEGSKO lens.

### • 3762

#### **Antioxidant delivery to the lens nucleus: A strategy to delay age related nuclear cataract**

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Age related nuclear (ARN) cataract is associated with the accumulative effects of oxidative damage to proteins in the lens nucleus. Since it is preceded by a loss of the key lens antioxidant GSH specifically in the lens centre, but not the lens periphery or cortex, ARN cataract is thought to be due to a failure to deliver GSH to the nucleus. Due to its large size, the avascular lens cannot rely on passive diffusion alone to deliver GSH to deeper lying fiber cells. Instead it has been proposed that the lens operates an internal microcirculation system which delivers nutrients to the lens nucleus faster than would occur by passive diffusion alone. Consistent with this model, our group has used MRI and confocal microscopy to visualize this microcirculation and have revealed that the lens sutures serve as an extracellular pathway to deliver molecules to the lens nucleus. Furthermore, we have shown that cells in the nucleus express transporters that are potentially capable of accumulating the GSH and glucose convected to them via the microcirculation system. These results suggest that harnessing the circulation system to deliver antioxidants to the lens nucleus is a potential strategy to delay the onset of ARN cataract.

### • 3763

#### **In vivo quantitative measurement of antioxidant effects in the lens**

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To evaluate the protective effect of antioxidants on experimental cataract induced by ultraviolet radiation (UVR).The protective effect of the antioxidants alpha-tocopherol and caffeine and the antioxidant gene Grx-1 were investigated. For investigation of the antioxidants, albino Sprague Dawley rats were used and for the Grx-1 gene, a Grx-1 knockout C57BL/6 mouse and its inbred wild type were used. As an oxidation insult, all animals were exposed to around threshold dose UVR in the 300 nm wavelength region, delivered during 15 min. The UVR was generated with a high pressure mercury arc lamp and the waveband was limited to around 300 nm. Dosimetry was assured with a calibrated thermopile. The rats and mice were sacrificed at 1 week and 48 hrs after exposure, respectively. Then, the lenses were extracted for forward light scattering measurement. In all three experiments, the animals were grouped at five different dose levels centered around the expected threshold dose. The Maximum Tolerable Dose-2.3;16 (MTD-2.3;16) was used as threshold estimate. The protection factor (PF) was calculated as the ratio between the threshold dose with protective factor and the threshold without the protective factor.The threshold dose for alpha-tocopherol was with and without 3.0 and 2.7 kJ/m<sup>2</sup>, respectively (PF= 1.1). The threshold dose for caffeine was with and without 5.7 and 4.6 kJ/m<sup>2</sup>, respectively (PF= 1.2). The threshold dose for Grx-1 was with and without 3.8 and 3.0 kJ/m<sup>2</sup>, respectively (PF= 1.3).As measured in vivo, caffeine has a more potent antioxidant effect after topical application than alpha-tocopherol administered parenterally. The Grx-1 gene provides the most potent antioxidant effect in vivo.

### • 3764

#### **Antioxidative properties of glutaredoxin 2 in the lens**

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Glutaredoxin (Grx) belongs to the oxidoreductase family that controls cellular redox homeostasis. The glutathione-dependent Grx has a typical CXXC motif at its active site for efficient antioxidative function, and contains cytosolic Grx1 and mitochondrial Grx2 isoforms found in the lens and other ocular tissues. Both Grx1 and Grx2 have dethiolase activity that can reduce oxidized thiols in proteins/enzymes and restore enzymes/proteins activities/functions. Grx2 has additional peroxidase activity that can eliminate reactive oxygen species to maintain redox balance in cells. Its ability to protect the mitochondrial function makes it an effective molecule in preventing cell apoptosis. Our studies have shown that knockout (KO) Grx2 gene in lens epithelial cells causes the cells to be less viable with high sensitivity to oxidative stress, leakage in mitochondrial membrane, low ability to detoxify H<sub>2</sub>O<sub>2</sub>, and apoptotic. Enrich Grx2 KO cells with native recombinant Grx2 protein can rescue the cells from oxidative damage and apoptosis. Grx2 KO cells show impaired complex I and complex IV functions with low ATP pool. Mice with Grx2 gene deletion develop age-related cataract much faster than that of the wild type control mice.

**• 3765****Age-related human nuclear cataract. Blindness due to inexorable protein deterioration***TRUSCOTT R**University of Wollongong, Illawarra Health and Medical Research Institute, Wollongong*

Nuclear cataract stems from the inexorable breakdown of long-lived macromolecules in the human lens. Although this realization is new, the overall framework is now quite clear. Racemisation, deamidation and truncation are the main drivers of protein denaturation and some amino acids are particularly susceptible to age-related decomposition. Understanding these processes leads to a conclusion that the prospects for reversing lens opacification are remote. Since age-related cataract appears to be inevitable, future strategies for slowing cataract formation may depend on a detailed examination of people who retain clear lenses into their eighth and ninth decades.

**• 3771****Macular nerve-fiber-layer measurement in early stage Alzheimer's disease using optical coherence tomography**

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**Purpose** Alzheimer's disease (AD) is characterized by brain neuronal loss resulting in brain atrophy. Post-mortem studies show a loss of retinal ganglion cells in this neurodegenerative disease. The purpose of this study was to compare thicknesses of the macular nerve-fiber layer (MNFL) by Optical Coherence Tomography (OCT) between early AD patients and control.

**Methods** Twenty GDS 4 AD patients and 25 controls were examined at Hospital Clínico San Carlos in Madrid (Spain). Patients underwent a complete ophthalmologic exam (visual acuity, refraction, color test, biomicroscopy, IOP, dilated fundoscopy, and OCT). OCT macular measurements were made by dividing the macula into 9 areas: i) a central area of 1mm radius (fovea); ii) two concentric areas of 3 mm and 6 mm each were subdivided in 4 regions: nasal, temporal, upper and lower.

**Results** In comparison with the control group, patients with early-stage AD presented a significant MNFL decrease ( $p < 0.05$ ) in the central area and in the 3 mm paracentral area. In the 6mm paracentral area statistically significant differences were restricted to the temporal region.

**Conclusion** In early-stage AD (GDS4), the MNFL was decreased. The measurement of macular thickness by OCT could be a complementary tool for early diagnosis and control progression of AD.

**• 3772****Age macular degeneration-alzheimer disease in-between correlations: One year screening, follow-up and outcome**

GONZALEZ C

FUTUROPHTA, Toulouse

**Purpose** To evaluate the evolution of the AMD-AD correlations and their in-between impact.

**Methods** PATIENTS: AMD patients: 240 patients, 3 Groups A, B, C. A: 70 AMD patients with first stage AMD.B: 64 Atrophy AMD patients with predominant atrophic areas, C:106 Neovascular AMD patients, with Neovascular AMD. AD patients: 240 patients, 4 groups.I:70 Normal,no significant patients;II:127 MCI patients,(4 subgroups: mild, moderate,intense,severe); III :29 Early AD, IV:14 AD. EXAM: ophthalmologic exam included ETDRS visual acuity (VA), complete ophthalmic examination, Fundus examination, autofluorescence imaging(FAF),(Region Finder Software,for atrophic areas),optical coherence tomography (Spectral Domain OCT) (OCT en face software), and fluorescein angiography (FA) and ICG when Neovascular complication. Cognitive evaluation is done with MMSE: Mini Mental State Examination (Folstein, GRECO), score allow to determine various groups and subgroups. Each AMD patient, would have besides cognitive evaluation, and each AD patients, ophthalmologic evaluation. Follow-up was done every 4 months.

**Results** Cognitive impairment differ in each AMD subgroup: Normal score: mild in group A(37%),more in group B(23%)than in group C(35%).MCI was predominant(A:63%,B:77%,C:62%)and the most in group B. Early stage AD:3% only in group C.After 1 year follow-up ,we observe more moderate cognitive impairment in Group B, few but the most severe worsening in Group C. AMD ophthalmologic signs are predictive and precursor for AD, and more than AD for AMD. Fundus examination and even more (FAF, OCT)are useful and needed to enhance AD screening and follow-up.

**Conclusion** The AMD-AD correlations are confirmed,enhance AMD as marker for AD,improve AMD and AD screening and knowledge.

**• 3773****MR imaging in posterior ischemic optic neuropathy**

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**Purpose** To present and explain the pathognomonic magnetic resonance (MR) findings in posterior ischemic optic neuropathy (PION).

**Methods** Three patients with the suspicion of PION underwent a MR examination of the anterior visual pathway.

**Results** There was a rapidly progressive unilateral (n=2) and bilateral (n=1) decline in visual function with deterioration of central visual acuity and an altitudinal visual field defect (n=2). Fundoscopy showed optic disc pallor after 6 weeks in all patients at the affected side. A relative afferent pupillary defect was seen in the patients with unilateral disease. The MR imaging findings revealed a central high signal in the chiasm (n=3) and intracranial portion of the both optic nerves (n=1)/ one optic nerve (n=2) with normal signal of the outer part of the chiasm/optic nerve.

**Conclusion** The pattern of the lesions can be explained by the blood supply of the optic chiasm which occurs through arterioles arising from the circle of Willis entering the chiasm and forming capillaries. In vitro research revealed a poorer capillary distribution in the central parts. The central high signal represents ischaemia due to the vulnerability of the central part of the chiasm/optic nerve. These findings are important as they are almost pathognomonic for ischemic changes in the anterior visual pathway. These findings are different from those reported in inflammatory/demyelinating disorders affecting the anterior visual pathway. PION should be differentiated from stroke in the posterior cerebral circulation territory because of the possible treatment with rt-PA in the latter. A dedicated imaging approach is necessary in patients with visual field loss of unknown origin in order to confirm or exclude ischemic changes of the anterior visual pathway.

**• 3774****Ophthalmological complications after treatment of carotid-ophthalmic artery aneurysms using flow diverter**

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**Purpose** To evaluate the ocular complications after insertion of a flow diverter "pipeline embolization device" (PED) to treat carotid-ophthalmic artery aneurysms. PED is an endovascular implant designed as a stent to alter blood flow into the aneurysm. Therefore it may decrease the blood flow in the ophthalmic artery, and induce ophthalmological complications.

**Methods** 22 patients suffering from carotid-ophthalmic artery aneurysm, treated by flow diverter were included. Every patient underwent retrospectively a complete ophthalmological examination including macular and optic disc OCT and visual field.

**Results** Among those 22 patients, 12 (54%) reported functional troubles: decreased visual acuity (6 patients), visual field loss (1), diplopia (1), amaurosis (1), photopsia or myodesopsia (3). 5 (23%) had clinical lesions: optic atrophy (3), distal embolism (2). No patient presented optic nerve compression after embolization as checked on MRI.

**Conclusion** After flow diversion insertion, ophthalmological complications are frequent and various. Ophthalmological complications are rather due to ischaemia than compression, and may lead to optic atrophy and loss of vision. However a majority of patients recover normal visual function

## • 3775

**Idiopathic optico-chiasmatic arachnoiditis (IOCA) – enigmatic or non existant disorder?**

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**Purpose** There was a plethora of publications on idiopathic optico-chiasmatic arachnoiditis (IOCA) between 1929 and 1980 but few if any subsequently, testifies to the paucity of cases in recent decades. Thus, the aim of the study was to analyse the original descriptions of the disease and verify its real nature.

**Methods** Study was based on the analysis of all historical descriptions of the diseases, including earliestarticles, textbooks, etc.

**Results** A 1929 report by the Argentine neurosurgeon Manuel Balado and his ophthalmic colleague Paulina Satanowsky is generally considered the first description of IOCA. By 1937 there were documented 129 surgically verified cases (13 associated with syphilis and the remainder idiopathic). The published descriptions of the disease was characterized by the great heterogeneity of the epidemiology, symptoms and signs. The decline of IOCA cases coincides with the advent of computerized tomographic scanning in the early 1970s. This has continued as more sophisticated imaging has been introduced.

**Conclusion** IOCA did not and does not exist as a separate and distinct clinical entity.

## • 3776

**Melanopsin retinal ganglion cells and circadian dysfunction in Alzheimer's disease**

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**Purpose** To evaluate optic nerve and in particular melanopsin retinal ganglion cells (mRGCs) in relation to rest-activity rhythm in Alzheimer disease (AD).

**Methods** Retinal nerve fiber layer (RNFL) thickness measurements by optical coherence tomography were performed in 21 AD and 74 age-matched controls. Actigraphic monitoring was performed in 16 AD patients and 10 age-matched controls. Non-parametric methods were applied to assess interdaily stability (IS), intradaily variability (IV) and relative amplitude (RA) of rest-activity rhythm. We also performed immunohistochemical analysis of mRGCs and axonal count on optic nerve cross-sections in 14 neuropathologically confirmed AD and 13 control post-mortem retinas.

**Results** OCT evaluation showed reduced average ( $p=0.03$ ) and superior ( $p=0.005$ ) RNFL thickness in AD patients. Actigraphic monitoring demonstrated an increased IV ( $p=0.04$ ) and reduced RA ( $p=0.04$ ) in AD. Furthermore, AD patients were significantly less active during the day ( $p=0.03$ ). Considering the patients with at least one circadian parameter outside the 2SD from the mean of controls, we found a significant correlation between IV, average ( $p=0.035$ ), superior ( $p=0.045$ ) and inferior ( $p=0.017$ ) RNFL thickness. Melanopsin RGCs density was significantly reduced, independently from age, in AD retinas ( $p=0.003$ ) and AD optic nerves showed variable degree of age-related axonal loss.

**Conclusion** We demonstrated a subclinical optic nerve involvement in AD patients, both by OCT and histopathology. We also documented rest-activity circadian dysfunction in AD patients. Post-mortem investigation revealed loss of mRGCs and RGCs with a different pattern in AD. The reduction of mRGCs may contribute to circadian rhythm dysfunction in AD.

**Industry-sponsored symposium 4: Non-infectious uveitis. A short look back and a long look forward****• 3821****Epidemiology, immunopathology, and the role of cytokines in non-infectious uveitis***NERIP  
Aguagliano***ABSTRACT NOT PROVIDED****• 3822****Classifying and stratifying patients to manage non-infectious uveitis and its complications***TAYLOR S***ABSTRACT NOT PROVIDED****• 3823****Where are we for treatment guidelines?***BODAGHI B  
Paris***ABSTRACT NOT PROVIDED**



**SATURDAY  
SEPTEMBER 21  
2013**

## Course 17: Pseudophakic retinal detachment

### • 4211

#### Vitrectomy for R.D. in eyes with different/unusual IOLs

GEORGALASIS  
Athens

Retinal detachment is one of the most serious complications of cataract surgery. Vitreous changes such as 'anterior movement' or 'protuberance of the posterior surface of the vitreous' have been postulated to occur, and cause posterior vitreous detachment which subsequently may lead to an increased incidence of retinal detachment in pseudophakic eyes. Additionally, several intraocular lenses(IOLs) such as multifocal, iris fixated or scleral fixated IOLs are more and more used by cataract surgeons. Retinal detachment in these eyes pose a new therapeutic challenge to vitreoretinal surgeons.

### • 4212

#### Problems in vitrectomy and gas for the treatment of Ps RDs

THEOCHARIS IP (1, 2)  
(1) Primevision.gr, Athens  
(2) Retina,Svetivid, Belgrade

The use of gas as a tamponade in vitreoretinal surgery is a major tool in the treatment of rhegmatogenous retina detachment (RRD), but it does not lack problems intraoperatively and postoperatively. The intraoperative problems include the risk of hypotony, poor visualization, dislocation of IOLs, rupture of zonules, the risk of compromising the choroidal and retinal circulation, the presence of a gas bubble in the anterior chamber. Postoperative problems are the lack of clear vision due to the gas bubbles/light reflections, the necessity of avoiding high amplitudes (ie flights), and may include high intraocular pressure, hypotony, inadequate filling of the vitreous cavity, dislocation of IOL, iris capture, contact of the gas with the cornea endothelium, dehydration of the retina, toxicity. Nowadays our experience in treating RRDs with gas, make the problems arising from its use rare and reversible and its use the most popular among the vitreoretinal surgeons and patients.

### • 4213

#### Pars Plana Vitrectomy alone vs Vitrectomy with scleral buckling for PRDs

ASTERIADISS  
Thessaloniki

Pseudophakic Retinal Detachment is a subgroup of Rhegmatogenous Retinal Detachments with some unique characteristics, such as the number and location of retinal breaks, certain intraoperative technical difficulties and possibly an increased risk of proliferative vitreoretinopathy. Recent evidence has established the role of pars plana vitrectomy as a primary method of managing these cases. The role of using scleral buckling techniques in combination with vitrectomy as well as the types of buckling that could be used remains controversial. At this talk, the above issues will be discussed in full, and a review of the literature as well as the personal experience of the speaker will attempt to clarify the role of using combined techniques in managing those cases.

### • 4214

#### Scleral buckle vs primary vitrectomy in PRDs

PARIKAKISEA  
Ophthalmiatrion Eye Hospital, Vitreoretinal Dpt., Athens

Pseudophakic retinal detachments seem to have a lower rate of surgical repair due to missed retinal breaks and increased risk of proliferative vitreoretinopathy. Scleral buckling is a practically unchanged low-budget procedure and was the gold standard in the past, but it involves ocular motility disturbances and refractive changes and needs certain surgical skills and experience. Primary small gauge vitrectomy in pseudophakia rapidly gains ground, as it is an easy, controlled surgical technique with less pain and the surgeon feels confident to have a complete intraoperative retinal attachment. In order to find the current role of conventional surgery in pseudophakic retinal detachments, scleral buckling has to be compared with vitrectomy alone as well as the combined procedure of vitrectomy plus encircling band, in terms of reattachment rate, functional outcome, reoperations and proliferative vitreoretinopathy incidence. Young patients, having clear lens extraction for myopia correction and post-traumatic cases with ora dialysis seem to be a reasonable group for conventional management.

**Course 17: Pseudophakic retinal detachment****• 4215****360 degrees prophylactic laser retinopexy during the surgery for the treatment of pseudophakic retinal detachment**

KOURENTIS C, XIROU T  
*Athens*

This is to identify whether 360 degrees prophylactic laser retinopexy during the surgery for the treatment of pseudophakic retinal detachment is predictive for the anatomical and functional outcome. May prophylactic laser treatment be protective for the occurrence of a retinal redetachment?

**• 4216****Macular problems in PsRD**

PAPPAS G  
*Venizeleio Hospital, Heraklion*

Post pseudophakic retinal detachment can lead to various macular problems. Post retinal detachment ERM, macular holes, folds involving the macula, submacular heavy liquid droplets and functional problems have been published. A presentation of these complications as well as techniques how to avoid them or to resolve is the aim of this presentation.

## • 4221

**Comparative study of the IOP-lowering effect of Xalatan vs generic latanoprost in normal subjects**

YIWEI (VINCENT) Q. POURJAVAN'S

*Cliniques Universitaires Saint Luc Ophthalmology, Woluwe Saint Lambert, Brussels*

**Purpose** To compare the intraocular pressure (IOP)-lowering efficacy of Xalatan with the generic latanoprost (Latanoprost EG) in normal healthy subjects.

**Methods** 20 normal subjects were recruited in this single-center, randomized, prospective, open label, two-period comparative study. At the baseline visit, subjects' baseline IOP was measured in both eyes at 8am in the morning. Subjects were then administered Xalatan in the right eye and Latanoprost EG in the left eye, both at 10pm, and IOP was controlled again the following morning at 8am. The student T-test was used to assess the level of IOP decrease significance in both groups.

**Results** The baseline IOP in all the 20 subjects was: . The IOP decrease in the first group administered with Xalatan was  $3.74 \pm 1.9$  mmHg ( $26.11 \pm 11.3$  mmHg). The IOP pressure at baseline and 10 hours after Xalatan administration were respectively  $14.33 \pm 2.1$  mmHg and  $10.59 \pm 3.9$  mmHg. In the second group, eyes administered with Latanoprost EG showed a slighter IOP decrease from baseline  $2.54 \pm 1.33$  mmHg ( $18.18 \pm 4.03$  mmHg). The IOP at baseline and 10 hours after Latanoprost EG were respectively  $14.01 \pm 2.37$  mmHg and  $11.45 \pm 2.39$  mmHg. This difference was significant. ( $p < 0.05$ )

**Conclusion** This study showed that the magnitude of IOP lowering in normal healthy subjects with Xalatan and Latanoprost EG is different, a higher IOP lowering efficacy of Xalatan compared to Latanoprost EG. This is important in Glaucoma patients especially when they are treated with different topical drops and for whom the maximum IOP decrease, achieving of the target IOP range, maintenance of the remaining visual field and quality of life is crucial.

## • 4223

**Does generic latanoprost measure up to the branded, well-known Xalatan?**

MARINESCU C, POURJAVAN'S

*Cliniques universitaires St. Luc, UCL, Ophthalmology, Brussels*

**Purpose** To compare the therapeutic efficacy of 0.005% Latanoprost versus Xalatan in the treatment of patients with OHT, POAG or secondary open-angle glaucoma.

**Methods** A prospective, single-center crossover, two period comparative study including 21 eyes of 13 patients. The patients from our glaucoma outpatient clinic, treated with Xalatan monotherapy were proposed to switch their treatment to 0.005% Latanoprost for 6 weeks. After 6 weeks, they were treated again in monotherapy with Xalatan. The IOP was measured during baseline (Xalatan1), after 6 weeks with the generic (Lat), and again after reintroducing the original medication, Xalatan for 6 weeks, (Xalatan 2). Two consecutive intraocular pressure (IOP) measures were taken during each control at 8 and 10 o'clock am. The primary end-point was the IOP variations between the brand name and the generic after the 12w. The Anova test was used to assess the difference.

**Results** The mean age was of  $67 \pm 13$  years. 7 men and 6 women were included in this study. The mean IOP at baseline was  $15.2 \pm 2.7$  mmHg with Xalatan. The mean IOP measurements after switching to Latanoprost and crossover to Xalatan were respectively  $17.6 \pm 3.5$  mmHg and  $15.3 \pm 2.6$  mmHg. There was, on average, an increase of IOP of  $2.89 \pm 1.2$  mmHg with Latanoprost. This difference was statistically significant ( $p < 0.05$ ).

**Conclusion** Our first results showed an increased of IOP in patients switching from Xalatan to Latanoprost. This is of major importance in treatment of the patients with glaucoma. The patients who are not accurately treated with one drop tend to have multiple drops which affects negatively the quality of life of the patient, the compliance, the persistence and causes neg

## • 4222

**Efficacy of a preservative-free fixed combination of bimatoprost and timolol in treatment-naïve versus previously treated patients**

CORDEIRO MF (1), GOLDBERG I (2), SCHIFFMAN R (3), BERNSTEIN P (3), LIU C (3), BEJANIAN M (3)

(1) *Western Eye Hospital, Imperial College Healthcare NHS Trust, London*(2) *Sydney Eye Hospital, University of Sydney, Sydney*(3) *Allergan, Irvine, CA*

**Purpose** To evaluate the intraocular pressure (IOP)-lowering efficacy of a preservative-free formulation of fixed-combination bimatoprost/timolol (FCBT PF) in treatment (tx)-naïve vs previously treated patients.

**Methods** A double-masked, randomized, parallel-group study compared FCBT PF with preserved FCBT (Ganfort) in 561 patients diagnosed with glaucoma or ocular hypertension who were tx naïve or had inadequate IOP lowering with previous tx. IOP was measured at 0, 2, and 8 hours at baseline and weeks 2, 6, and 12. Subgroup analysis evaluated average IOP change from baseline in tx-naïve vs previously treated, washed out patients.

**Results** FCBT PF was equivalent to Ganfort, producing numerically greater (0.2 to 0.5 mmHg) IOP lowering than Ganfort during follow-up. With FCBT PF, IOP mean changes from baseline (mmHg) ranged from  $-8.7$  to  $-9.8$  in tx-naïve patients (n=50) and  $-7.3$  to  $-8.5$  in previously treated patients (n=228). Baseline IOP, age, diagnosis, and corneal pachymetry were significant covariates of IOP lowering. In an analysis of covariance model with these factors as covariates, differences between tx-naïve and previously treated patients ranged from 0.8 to 1.7 mm Hg favouring tx-naïve patients and were significant ( $p=0.018$ ) at 7 of 9 timepoints.

**Conclusion** FCBT PF had a favourable IOP-lowering effect compared with Ganfort and reduced IOP more effectively in tx-naïve than previously treated patients. This can be attributed in part to the removal of preservative (BAK) optimizing the efficacy of timolol in FCBT PF.

*Commercial interest*

## • 4224

**24h IOP control by subchronic AMA0076 administration in Dutch Belted rabbits**

VAN DE VELDES S (1), VAN BERGEN T (1), HOLLANDERS K (1), SIJNAVE D (1), VANDEWALLE E (2), MOONS L (3), STALMANS I (1, 2)

(1) *KU Leuven Ophthalmology, Leuven*(2) *UZ Leuven Ophthalmology, Leuven*(3) *KU Leuven Biology, Leuven*

**Purpose** To investigate IOP lowering efficacy of rho kinase (ROCK) inhibitors, AMA0076 and Y-39983, after subchronic treatment.

**Methods** Dutch Belted rabbits (5 rabbits/concentration) were treated daily with a single topical dose of AMA0076 (0.05, 0.1 and 0.4%) or Y-39983 (0.025, 0.05 and 0.1%) in a masked fashion for 14 days. Saline was used as control in the contralateral eye. IOP was measured before, 3 and 7 hours after administration of the treatment. AMA0076 is a locally acting ROCK inhibitor, to reduce side effects such as hyperemia otherwise seen after administration of a ROCK inhibitor.

**Results** Mean baseline IOP before the start of the experiment was  $21.02 \pm 0.42$  and  $20.69 \pm 0.39$  mmHg for AMA0076 and Y-39983, respectively. Significant IOP reduction was observed in all AMA0076 and Y-39983 treatment groups. A maximal IOP reduction of 39 and 35% was reached with AMA0076 0.1% and Y-39983 0.05%, respectively. Initially, IOP returned to baseline values 24h after administration of AMA0076. After 3 days of treatment with AMA0076 a sustained IOP lowering effect was present, IOP did not return to baseline values, but remained significantly lower for all concentrations of AMA0076 ( $p < 0.05$ ). Treatment with Y-39983 did not show this sustained IOP decrease. As a result of this sustained IOP lowering effect smaller IOP fluctuations were observed in animals treated with AMA0076 compared to Y-39983. Hyperemia was observed with all concentrations of Y-39983 whereas only mild hyperemia was induced after administration of AMA0076 0.4%.

**Conclusion** Once daily treatment of rabbits with ROCK inhibitor AMA0076 resulted in IOP reduction that was more sustained and associated with smaller peak-through fluctuations than Y-39983.

• 4225 / T045

**Patient behavior when prescribed non-affordable glaucoma medication in the medical unit, National Research Center, Cairo, Egypt**

SAEED IBRAHIM A

Ophthalmology department, National Research Center, Cairo

**Purpose** (1)Identify cost-reducing strategies,including cost related non-adherence(CRN),employed by primary open angle glaucoma (POAG)patients;(2) explore impact of doctor-patient interaction(DPI)on CRN and(3)pinpoint best DPI methods that minimized CRN.

**Methods** A Cross-sectional study was enrolled on consecutive adult patients attending the eye clinic of the National Research Center,Cairo,Egypt, diagnosed with POAG and on outpatient topical anti-glaucoma drugs in the first 8 months of 2012.Data were collected via an interviewer-administered questionnaire.The impact of factors as education,insurance and drug cost to income ratio were tested statistically.

**Results** Data gathered from 280 patients showed that 75% of patients employed at least 1 method of CRN(termed CRN+).Of those, up to 70% skip doses,64% postpone buying prescriptions and 28% ignore buying it altogether.We found significant relationships between lower CRN scores and better insurance coverage( $p=0.002$ ),higher education( $p=0.002$ ),lower values of "drug cost to monthly income" ratio( $p=0.003$ ),lower number of prescriptions( $p=0.003$ )and better DPI regarding drug costs( $p=0.004$ ). 58% of CRN+ patients reported having DPI,(termed CRN+DPI).In CRN+DPI, the most common methods were doctors showing sympathy towards patients' drug costs(72%),ensuring patients' affordability of drugs(65%)and change one to a cheaper alternative(62%).In those who did not have DPI,the most cited cause was "being embarrassed" to ask about drug costs(80%).

**Conclusion** Future policies should focus on improving DPI to lower CRN,mainly,ensuring patient education on drug costs, showing strong emotional support and reducing polypharmacy. Encouraging patients to ask for cheaper alternatives can also help.

• 4226 / T043

**Removal of preservative from Ganfort improves intraocular pressure (IOP) lowering in patients – A timolol dose-response phenomenon**

SHEN J (1), BEJANIAN M (2), SCHIFFMAN R (2)

(1) *Pharmacokinetics and Drug Disposition, Irvine*

(2) *Clinical Development, Irvine*

**Purpose** A benzalkonium chloride (BAK)-free fixed-combination formulation of bimatoprost and timolol (Ganfort) has been developed for patients who are sensitive to preservatives. A randomized controlled study in 561 patients compared the IOP-lowering efficacy of the BAK-free formulation versus the preserved Ganfort. At all timepoints, IOP lowering, numerically, consistently favoured the BAK-free formulation. This finding contradicted expectations that the removal of BAK would result in lower ocular bioavailability of both bimatoprost and timolol, resulting in less efficacy. Research was conducted to explore possible explanation for the clinical observation.

**Methods** Literature search on timolol dose response was performed, focusing on IOP lowering following topical dosing of 0.1%, 0.25%, and 0.5% timolol ophthalmic solutions.

**Results** A U-shaped dose-response curve exists for timolol, and available data suggest that the dose that elicits maximal IOP lowering lies between 0.25% and 0.5%.

**Conclusion** Removal of BAK from the fixed combination product Ganfort, which contains 0.5% timolol, may have resulted in a lower, but optimal, ocular concentration of timolol, leading to improved IOP-lowering efficacy with the preservative-free formulation.

**Commercial interest**

• 4227 / T044

**SYL040012, a siRNA for the treatment of glaucoma**

PAÑEDA C

R&D, Tres Cantos, Madrid

**Purpose** SYL040012 is a siRNA under development for the treatment of glaucoma and increased intraocular pressure (IOP). siRNAs are small double stranded RNAs that regulate protein expression at transcription level using the endogenous RNA interference machinery. Therapeutically siRNAs are attractive due to their specificity, potency and ability to silence targets that are not addressable by small molecules. SYL040012 targets ADRB2, a well-known target for glaucoma.

**Methods** Silencing activity of SYL040012 was validated in cell lines and IOP lowering efficacy was studied in rabbit. Silencing activity was assessed by extracting total RNA from cells or animal tissues and analyzed by real time-PCR. IOP was measured using applanation tonometry. Tissue and plasma exposure was evaluated following a single instillation to rabbits using a non-denaturing Anion Exchange HPLC combined with fluorescence detection. 28-day regulatory toxicology studies were performed in cynomolgus monkeys following a daily ocular instillation of one of three doses of SYL040012 or vehicle. Phase I clinical trials were performed in healthy volunteers or individuals with increased IOP that received either a single dose or seven repeated administrations of SYL040012.

**Results** SYL040012 reaches structures of the eye relevant to the treatment of glaucoma when administered in eye drops; but is only detected in systemic circulation and non-ocular tissues at trace levels. Presence of this compound in the ciliary body it results in specific down-regulation of the target gene and reduction in IOP in animal models. Preclinical and clinical studies show that SYL040012 is well tolerated both systemically and locally.

**Conclusion** In summary, SYL040012 is a promising candidate for the treatment of increased intraocular pressure associated to glaucoma.

**Commercial interest**

• 4228 / T067

**Validation of Testvision, an internet-based test for the detection of visual field loss**

OLSEN AS (1), LA COUR M (1), SERUP L (2), ALBERTI M (1), DAMATO B (3), KOLKOM (1, 4)

(1) *Department of Ophthalmology, Glostrup University Hospital, Copenhagen*

(2) *Private Ophthalmologist, Copenhagen*

(3) *UCSF, San Francisco*

(4) *Department of Neuroscience and Pharmacology, University of Copenhagen, Copenhagen*

**Purpose** The aim of the present study was to determine if the program Damato Testvision (DT), www.testvision.org, adequately detects visual field loss from glaucoma.

**Methods** DT is available in three versions and was tested on 97 patients (188 eyes). The procedure was performed with a laptop connected to a computer monitor and a mouse placed on a height adjustable table. To determine sensitivity and specificity DT was compared to two gold standards: 1; Hodapp, Parrish and Anderson classification (Hodapp) of Humphrey 30:2 and 2; doctors' diagnoses.

**Results** A total of 361 DT Standard tests were performed on 115 eyes. DT comparisons with Hodapp achieved sensitivity and specificity of 55% and 93% respectively when comparing no disease to early glaucomatous loss (AUC; 0.871). If early loss was merged with the no disease group, the sensitivity and specificity were 78% and 90% respectively (AUC; 0.931) Compared to the doctors' diagnosis DT achieved sensitivity and specificity of 52%; 96% respectively when borderline patients were classified in the glaucoma group (AUC; 0.867). If borderline patients were classified as controls: DT achieved sensitivity and specificity of 63%; 92% (AUC; 0.897).

**Conclusion** Our study indicates that DT may be a promising tool to detect visual field defects in a pre-selected population. The test is successful without prior training of the test person but minimal computer knowledge is advisable. The authors believe that internet based visual field examination may play a role as an easy accessible and affordable test. With further development, DT could be a tool for glaucoma screening in the home setting. Future studies need to evaluate DTs performance in the general population without prior selection.

**• 4231****Biomaterials for ophthalmic implants - all for one and one for all?**

STORSBERG J (1), SCHMIDT C (1), FUCHSLUGER T (2), WEBER A (3), SEL S (4)  
 (1) Potsdam  
 (2) Düsseldorf  
 (3) Stuttgart  
 (4) Heidelberg

Having one biomaterial for a wide range of clinical applications may mean that the physico-chemical properties of the material surface needs to be tailored to fulfill the all requirements of the actual application. One example of tailoring surface properties of one and the same base material may include the definition of areas where cells attach to and deposit extracellular matrix. As for ophthalmological applications, this notion can be traced to de Quenngy's review, published in 1789. There, the author outlined basic features of the keratoprosthesis: a central optical cylinder and a haptic for firm positioning in the surrounding tissue. Incomplete biocompatibility of materials used, aside from issues of ethics and patient's acceptance, render problems. Using chemical, physical and nanotechnological methods, features of the material can be dramatically increased. There are different strategies to induce biomimetic cell-tissue-material interaction by modifying the surface on one base material – as well as the use of metallic biomaterials and drug-containing particles. These findings aid the development of next-generation biomaterials where surface-effects are utilized in different therapeutic clinical applications.

**• 4232****The LentiVector® gene therapy platform for ocular disease: A clinical update**

ELLIS S  
 Oxford

The LentiVector® Gene Therapy Platform for Ocular Disease: a clinical update Oxford BioMedica currently has three ocular therapies in clinical development using the LentiVector® platform: RetinoStat®, StarGenT and UshStat®, for the treatment of age-related macular degeneration, Stargardt macula dystrophy and Usher Syndrome 1B respectively. A clinical update of these trials will be given.

**• 4233****Nanoparticles for drug delivery to the retina**

STEFANSSON E  
 Reykjavik

We have developed a drug delivery platform based on cyclodextrin nanoparticles in eye drop suspensions. The nanoparticles increase drug solubility, extend mucoadhesion and penetration into the eye. High concentrations of drug in the tear film for extended periods of time allow for greatly increased penetration into the eye and relatively less washing into the nasolacrimal apparatus. Dexamethasone nanoparticle eye drops are effective against diabetic macular edema and uveitis. Cyclodextrin nanoparticle eye drops allow drug treatment of retinal disease with eye drops.

**• 4234****Nanoparticles for delivery of therapeutical nucleic acids to corneal endothelium**

CZUGALA M (1, 2, 3), MYKHAYLYK O (4), SINGER BB (1), STEUHL KP (5),  
 ERGÜN S (6), WESSELBORG S (2), EPPLER M (7), PLANK C (4), FUCHSLUGER T (3)  
 (1) Institute of Anatomy, University Hospital Essen, Essen  
 (2) Institute of Molecular Medicine, University Hospital Düsseldorf, Düsseldorf  
 (3) Department of Ophthalmology, University Hospital Düsseldorf, Düsseldorf  
 (4) Institute of Experimental Oncology, Technical University Munich, Munich  
 (5) Clinic for Diseases of the Anterior Segments of the Eyes, University Hospital Essen, Essen  
 (6) Institute of Anatomy and Cell Biology, University of Würzburg, Würzburg  
 (7) Institute of Inorganic Chemistry, University Duisburg-Essen, Essen

Corneal endothelium is as crucial for maintenance of corneal function as difficult it is to be accessed with any treatment that doesn't involve invasive surgical procedures. However it is possible to administer therapeutic agents by topical application, their effect on deeper corneal layers and further fate remains of our control. This study has been designed to employ nanoparticles in order to improve our chances in therapy of corneal dystrophies as well as to prevent endothelial cell loss after transplantation of the cornea. Variety of nanoparticles serves a potential solution to problem of targeting the corneal endothelium, when properly functionalized to assure precise targeting. Widely proven as efficient carrier for nucleic acids may enable any gene therapy or gene silencing to be conducted with minimized risk of uncontrolled transfection. When to concern both growing need for corneal transplants and shortage of tissue, efficient therapy to protect graft from failure or even to protect patient's own cornea becomes essential.

**• 4235****Nanoparticles incorporated collagen hydrogels for sustained release of EGF**

RAFAT M (1, 2), MONDAL D (2), ISLAM M (2), LIEDBERG B (2), GRIFFITH M (2)

(1) Biomedical Engineering, Linköping

(2) Clinical and Experimental Medicine, Linköping

Therapeutic biomolecules such as growth factors are essential for enhancing the regeneration of damaged tissues by inducing cell signalling activities such as cell migration, proliferation, and differentiation. Nevertheless, they have short half-lives in physiological conditions due to fast deactivation and degradation by enzymes and other physical and chemical reactions. Therefore, there is a great need for the suitable target delivery of nanoparticles to improve the release kinetics of growth factors as well as their therapeutic effectiveness. The main objective of this study was to develop and characterize a sustained delivery system consisting of an EGF-encapsulated chitosan nanoparticles and collagen hydrogel carrier system to achieve a sustained release of EGF. In this study, we made EGF-loaded chitosan nanoparticles, which could be incorporated into an engineered collagen hydrogel scaffold. The particles were spherical in the size range of 60–100 nm. The release kinetics of EGF showed the release of growth factors in a sustained manner. Live-dead staining of human corneal epithelial (HCEC) cells was done to evaluate the cytotoxicity of the nanoparticles.

**• 4241****The treatment of retinoblastoma with intravenous chemotherapy + local treatment**

*DESIARDINS L, LUMBROSO LEROUIC L, LEVY C, CASSOUX N, AERTS I,  
SAVIGNONI A, DENDALE R, SASTRE X  
Paris*

**Patients and method:** Treatments protocols applied since 1995 included intraveinous chemotherapy at various dosages with local ophthalmological treatment by transpupillary thermotherapy, cryotherapy and iodine 125 plaque brachytherapy. The data concerning initial findings, treatments used, and results are registered in our database in a prospective way. We performed a retrospective study of children treated between January 1995 and December 2009.**Results:** There were 317 patients with bilateral retinoblastoma and 411 patients with unilateral retinoblastoma. The median follow up is 72 months. Median age at diagnosis was 7 month for bilateral and 20 months for unilateral. In bilateral patients, 104 had conservative management of both eyes, 204 had a conservative management of one eye and enucleation of the fellow eye and 9 had bilateral enucleation. Among the 312 eyes treated conservatively, 83 eyes were irradiated and 13 had secondary enucleation. (Success rate 70%) Among 411 unilateral retinoblastoma, 87(21%) were treated conservatively. 71 eyes were preserved (67 without external beam and 4 with external beam) (success rate 77%).**Conclusion:** Chemotherapy associated to local ophthalmological treatments is efficient in the

**• 4242****Intravitreal chemotherapy: Indications and results**

*MUNIER F (1), GAILLARD MC (1), BALMER A (1), BECK-POPOVIC M (2)  
(1) Jules-Gonin Eye Hospital, Lausanne  
(2) CHUV, Lausanne*

The presence of active vitreous seeding following state-of-the-art conservative treatment of intraocular retinoblastoma leads in the vast majority of cases to either external beam irradiation (EBR) and/or enucleation. Intravitreal chemotherapy (IVC) is theoretically the best route for delivering the highest vitreous concentration of a chemotherapeutic drug, over intravenous, periocular and ophthalmic artery chemotherapy. This makes IVC the best candidate for treatment of diffuse vitreous disease in eyes with retinoblastoma, but it has remained highly controversial, as considered an invasive procedure associated with the risk of tumor exteriorization. We retrospectively reviewed all consecutive patients with vitreous disease found eligible and then treated by intravitreal injections of melphalan. IVC was performed every 7 to 10 days up to 8 injections of Melphalan (20-30 mg) per event, using a novel injection technique characterized by the prevention of vitreous reflux and sterilization of the needle track. The results appear promising in terms of safety (with no evidence of exteriorization), and efficacy with unprecedented salvage rate over 85%. Control of vitreous seeding was achieved without EBR in all cases.

**• 4243****Intraarterial chemotherapy: Indications and results**

*HADJISTILIANOU T  
Sienna*

IA chemotherapy is a promising primary and salvage treatment for moderate and advanced RB particularly in eyes designed for enucleation. Indications include: Primary treatment of unilateral retinoblastoma Reese-Ellsworth Stage II, III, IV, Va & Vb (ABC Stage B, C & D); adjunctive treatment after systemic chemotherapy, with or without focal therapy; incomplete response to conservative therapy; local tumor recurrence after conservative therapy. Most of adverse side effects are transient, choroidal vasculopathy is permanent and in some cases progressive. Intra-arterial chemotherapy conserves many eyes with retinoblastoma that would otherwise require enucleation, at the same time avoiding the risks of radiotherapy and systemic chemotherapy.

**• 4244****Management of locally advanced retinoblastoma**

*LUMBROSO L  
Paris*

**ABSTRACT NOT PROVIDED**

• 4245

**Case reports**

*DESJARDINS L, MUNIER F, HADJISTILIANOU T, LUMBROSO L  
Paris*

**ABSTRACT NOT PROVIDED**

## SIS: Optical and functional correlation of forward and backward scattering in the lens

### • 4251

#### Basics of forward and backward scatter in the human eye lens

VAN DEN BERG TJTP

*Netherlands Inst Neurosc., Royal Academy, Amsterdam*

This presentation will treat basics of light scattering from the physical point of view, and as applicable to the human eye lens. The type of light scattering most easily recognized in ophthalmology is volume scattering, i.e. the more or less uniform lightness as seen in the slitlamp image. In fact this seeming uniformity derives from light scattering by a dense distribution of small particles. The ratio between backward and forward scatter strongly depends on particle size. Particles much smaller than wavelength scatter about equally in forward and backward directions; larger particles scatter (much) more strongly in forward direction. The particles dominating backward scatter in the human eye lens (towards the slitlamp, scheimpflug camera, etc.) are much smaller than wavelength. The particles dominating forward scatter have sizes of the order of wavelength. So, no direct link exists between slitlamp observation and hinder to the patient, as can be assessed with straylight. However statistically, the amounts of both types of scatterers can be linked. The less easily observed and understood type of scatter is surface scatter, e.g. from "Wasserspalten". Optical bench study of human eye lenses on these phenomena will be presented.

*Commercial interest*

### • 4252

#### Lens slitlamp image grading and nuclear hardness

BARRAQUER R

*Institut Universitari Barraquer, Barcelona*

Lens slitlamp image grading and nuclear hardness  
**Purpose:** To develop a lens nuclear grading system able to predict the hardness or surgical difficulty during cataract surgery.  
**Methods:** A 1 to 10 (plus 0) grading of the lens nuclear "density", thus called BCN-10 was developed based on standardized clinical slit lamp photographs, from complete transparency (0) to a totally black cataract (10). The BCN-10 scale was tested for intra-observer and inter-observer repeatability, for correlation with forward scattering measurement (C-Quant) and surgical difficulty (ultrasound time).  
**Results:** BCN-10 grading shows good intraobserver and interobserver repeatability, good correlation with surgical ultrasound times and fair correlation with forward scattering (glare) measurements.  
**Discussion:** Available lens grading systems as the LOCS III do not include the harder grades of cataract, precisely where prediction would help the surgeons. BCN-10 is a novel and consistent lens nuclear grading system useful to predict the hardness and difficulty that will be encountered during cataract surgery, and to some extent correlates with cataract-induced glare disability.

### • 4253

#### Pentacam HR and C-Quant – forward versus back-ward light scattering, what is the correlation?

WEGENER A

*Department of Ophthalmology, University of Bonn, Bonn*

A pilot study with 20 patients of varying age and sex has been conducted to compare light scattering measurements performed with the Pentacam HR and the C-Quant in patients with various ocular problems. The Pentacam HR measurements were done in mydriasis with the standard 25 image recording process. The C-Quant measurements were done in miosis according to the manufacturer's instructions. Pentacam images were exported for densitometric analysis to a custom-made software, whereas the C-Quant data were evaluated with the instrument software. During normal, age-related light scattering increase, C-Quant data were less sensitive for the detection of scattering changes than densitometric evaluation of the Scheimpflug images. However, certain disease conditions like diabetes evidenced a sensitive difference between the 2 measurement techniques.

### • 4254

#### Pentacam measurements and IOL scattering

BALIMEISTER M

*Goethe University, Frankfurt*

Rigid and foldable intraocular lenses of various materials have shown light scattering phenomena such as surface light scattering and glistenings. The impact of these phenomena on visual function is under controversial discussion. The scattering of light in an IOL can be measured in various ways. In many studies subjective grading at the slit-lamp has been used to quantify the intensity of scattering. More recently, evaluation of high-resolution digital Scheimpflug images has been able to provide an objective assessment of scatter and material inhomogeneities. Better quantification of these phenomena may achieve more meaningful correlations to results of functional measurements.

**• 4261****Cataract surgery up to the age of Daviel**

DE LAEY JJ

*Ophthalmology, Ghent University Hospital, Ghent*

The knowledge of ocular anatomy in the classical time was limited. Aristotle considered the lens as a post-mortem artefact. For Celsus the lens was the essential organ of vision, a misconception which will remain till Vesalius, one of the first to consider its optical role. Platter recognized the role of the retina and Aquapendente will correct the position of the lens in the eye, which was thought to be in its centre. Cataract was considered an accumulation of "miasma" between the lens and the iris. It is not certain that the ancient Egyptians performed cataract surgery. It was first mentioned by Sushutra, the father of Indian surgery. His technique reached Rome probably via Alexandria. The description of couching by Celsus corresponds to 17th century descriptions of the procedure. Till the late 18th century this was often performed by itinerant cataract surgeons. One of the most notorious of these quacks was Chevalier Taylor who blinded Bach. Because Daviel, as others, experienced failures with couching, he introduced the corneal incision and expression of the lens by gentle pressure. This revolutionized cataract surgery although couching was still used in a number of centres till the middle of the 19th century.

**• 4262****Who really introduced pneumatic retinopexy for retinal detachment outpatient management?**

ASCASO F

*Department of Ophthalmology, "Lozano Blesa" University Clinic Hospital, Zaragoza*

The history of Ophthalmology recognizes Dr. George F. Hilton as the first author to report a series of retinal detachments treated by pneumatic retinopexy (Ophthalmology 1986 May;93(5):626-41). Nevertheless, one and a half years before, Dr. Alfredo Dominguez published in Spanish two papers about gas surgery used in the office to treat "conventional" cases of retinal detachment (Arch Soc Esp Oftalmol 1985;48:47-54 and Studium Ophthalmologicum 1985, 2º época;IV:11-18). Our purpose is to give him his due and return his scientific priority.

**• 4263****Controversies in the history of glaucoma**

GRZYBOWSKIA (1,2)

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There are several interesting controversies in the history of glaucoma. The first corresponds to the origin of its name. It was argued that the ancient Greek word *glaukos* meant: 1. "to glow" or "to shine"; 2. "blue-white" or "blue-green"; 3. dull sheen or "glaze" of blindness; 4. owl: "glaucomati" meaning "having the eyes of an owl"; 5. a mortal fisherman who was transformed into a sea-god after eating a magical herb. The second controversy corresponds to the meaning of the word glaucoma. Several ancient authors, including Celsus, Roufos of Ephesus and Galen, described glaucoma as a disease of the crystalline, which was transformed to glaucos because of moisture. This understanding was continued in the 18th century by Banister, Platner and Boerhaave, who argued that glaucoma was a malignant form of cataract that begins with acute pain and terminates in amaurosis. The third controversy corresponds to the different concepts of glaucoma etiology in the 19th century, including gouty iritis, serous choroiditis, thickening of the sclera, irritation of the secretory nerves of the eye, a disease of the vitreous, and "secretory neurosis" (the primary cause of is the rise in pressure).

**• 4264****Trichromasy versus colour opponency: The controversy in 19th and 20th century's colour science and its solution**

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(1) *Dept. of Ophthalmology, Mannheim Faculty of Medicine, University of Heidelberg, Mannheim*(2) *General Hospital, Nesvizh*(3) *Darmstadt*

Purpose: To recall in mind one of the most dedicatedly fought discussions in visual science: that about trichromatic versus colour opponent organization of human colour vision. Methods: Literature survey, personal communication Results: In 19th and early 20th century, the community of colour scientists was divided into followers of Young's & Helmholtz's trichromatic theory, and followers of Hering's colour antagonistic concept. Both parties disposed on convincing arguments. Both confessions (as one is tempted to call them) felt that their particular belief necessarily had to be the truth. Both, in fact, contained truth. The controversy eventually found its solution in the zone theory of v. Kries which subsequently has received overwhelming experimental proof: trichromasy at receptoral level, colour opponency throughout the postreceptoral network. Conclusion: Browsing through early colour science discloses surprising ad captivating developments. One is in particular satisfying: the controversy concerning trichromatic versus colour antagonistic organization of colour vision. Eventually both antitheses were proven true and combined to the synthesis of zonal organization of colour vision.

**• 4265****Description of retinoblastoma by Pieter Pauw of Leiden in early 1600's***KIVELÄ T**Department of Ophthalmology, Helsinki University Central Hospital, Helsinki*

In 1963, Edwin B. Dunphy of Boston credited the 16th century Dutch anatomist, Pieter Pauw (1564-1617) of Leiden, with the first literary description of retinoblastoma made in 1597. Dunphy believed that the description referred to an advanced retinoblastoma that had broken into the orbit and temporal area. This opinion and credit is repeated often even in recent literature, in spite of the fact that a critical translation of the original Latin text of Pauw suggested in 2003 that another orbital tumor, such as embryonal rhabdomyosarcoma, would equally well, if not better, explain Pauw's observations. This presentation will review the evidence for and against retinoblastoma in the autopsy report. Retinoblastoma is likely to be as old as humankind, and exactly when this fascinating tumour first appeared in historic records will remain a matter of dispute.

**• 4321****Molecular sensors for the decoding of homeostasis disruptions  
in the retinal pigment epithelium: towards the understanding of  
retinal degenerative diseases***BAZAN**Neuroscience Center of Excellence and Department of Ophthalmology, School of  
Medicine, Louisiana State University Health Sciences Center, New Orleans*

The significance of the selective enrichment in omega-3 essential fatty acids DHA, docosahexaenoic acid) in photoreceptor cells has remained, until recently, incompletely understood. We contributed to the discovery of a docosanoid synthesized from DHA by 15-lipoxygenase-1, which we dubbed neuroprotectin D1 (NPD1: 10R,17S-dihydroxy-docosa-4Z,7Z,11E,13E,15E,19Z hexaenoic acid). NPD1 is promptly made on demand when homeostasis is at stake, as in oxidative stress ,proteostasis dysfunctions and in early stages of neural injury, ischemia-reperfusion or neurodegenerations. Thus NPD1 is a protective sentinel, one of the very first defenses activated when cell homeostasis is threatened . The availability of anti-apoptotic BCL-2 proteins is positively modulated by NPD1, whereas pro-apoptotic BCL-2 proteins are negatively regulated, as is activated microglia. Neurodegenerative diseases, in addition to enhancing oxidative stress, disrupt the proteostasis network and leads to a cellular inability to scavenge structurally damaged abnormal proteins. The RPE cell response cascade potentiates disruptors of homeostasis through multiple checkpoints and signaling networks. NPD1 , a key component of this response and of the lipidomic signature, targets neuroinflammatory signaling and proteostasis and in turn promotes homeostatic regulation of the transcription of key genes that in turn act as molecular decoders of input, and thus results in cell survival. (Supported by NIH: NINDS R01 NS046741, NEI R01 EY005121)

**SIS: Bench to bedside to registration - the intricate world of drug development****• 4411****Introduction - The challenges to drug approval: Going from an idea, early data onto registration***DESMET MD**MIOS Retina and Uveitis center, Lausanne*

Purpose: Gain a better appreciation of the steps involved in drug/device development and registration. Specific aim of this segment: The first challenge is to decide whether or not a drug or device is worth investigating further. Obtaining sufficient data and keep the process attractive to potential investors is a challenge as academics face a need to publish, thereby securing additional funding, while investors want to preserve intellectual property. One year after publication or presentation, data referring to a specific compound, process or device is considered to be part of public domain and cannot be patented. Prior to approval, a well defined package of efficacy, safety, and purification/production characteristics are required under good laboratory practice (GLP) guidelines. The clinical trial phase presents its own challenges. The desire to establish rapidly proof of concept, dose and frequency of administration often leading to premature costly choices, or overly complex study designs. The need to recruit within short timelines, increases the risk of inappropriate patient selection criteria. This lecture and others in this symposium, an insight into drug development and its intricacies will be presented.

**• 4412****Pre-clinical testing: Good laboratory practice and the requirements imposed by the agencies***VEZINA M**Ocular and Neuroscience, Senneville*

In all countries, preclinical (nonclinical) studies are required to support testing of novel therapeutics in humans. For ocular therapeutics there is no defined guideline available from the major regulatory authorities. This combined with a wide variety of compound types and delivery systems can make it challenging to determine what nonclinical studies should be conducted to support the human clinical trials. The therapeutic indication, class of compound, species specificity and suitability, dose route, systemic exposure, whether the compound is a new molecular entity or an already approved product and the intended clinical protocol, all have an impact on the nonclinical ocular program design. This presentation will outline the types of nonclinical studies that are typically appropriate for ocular therapeutics in the context of the previously mentioned considerations and the role of Good Laboratory Practice (GLP) and regulatory agencies in the design and acceptance of these studies.

***Commercial interest*****• 4413****Designing clinical trials - How best to optimize ideas, resources and patients***LEHMACHER W**Köln***ABSTRACT NOT PROVIDED****• 4414****Running clinical trials - The challenges of international research***ANGLADE E**Jersey City, NJ***ABSTRACT NOT PROVIDED**

• 4415

**Facing the regulators - Partnership or opponent**

*GRANZER U  
München*

**ABSTRACT NOT PROVIDED**

• 4421

### Glaucoma surgery outcome in Rwanda

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(1) Kabgayi Eye Unit, Muhanga

(2) Ophthalmology, Mechelen

**Purpose** To assess long term intraocular pressure (IOP) outcome after adult trabeculectomy surgery in Africa.

**Methods** All adult glaucoma patients who underwent trabeculectomy surgery in the Kabgayi Eye Unit, Rwanda between August 2003 and March 2008 were invited for a follow-up visit. Surgical and clinical data were collected from medical records. At the study visit best corrected visual acuity was measured and Goldmann applanation tonometry and biomicroscopy were done. Good IOP outcome was defined as both an intraocular pressure  $\leq$  15mmHg and achieving  $\geq$  30% reduction from the preoperative IOP. Odds ratios for good IOP outcome were calculated for potential predictors. For the first eyes multivariable analysis was done using logistic regression, as well as Kaplan-Meier survival curve analysis.

**Results** Participation rate was 74.2% (118 patients, 161 eyes). Preoperatively, the mean IOP was 31.2mmHg (SD 10.7, range 12-60). Anti-metabolites were applied during the operation in 79.5% of eyes. At the time of the follow-up study visit the mean postoperative IOP was 13.0 mmHg (SD= 5.1, range 4-35). A good IOP outcome was achieved in 66.9% (n=105) of eyes. Trabeculectomies using mitomycin C were 3 times as likely to have good IOP outcome than without (OR=3.0, CI= 1.2-7.1, P=0.008). The cumulative probability to maintain good IOP outcome reduced from 75% at a follow-up of 2 years to 25% after 5 years. At multivariable analysis long duration of follow-up (OR= 0.31, CI= 0.11-0.7, P= 0.009) and the presence of an elevated bleb (OR= 3.2, CI= 1.3-8.1, P= 0.012) remained significant predictors.

**Conclusion** Trabeculectomy with mitomycin C is effective in lowering IOP in Central Africa. However, the IOP control reduced at a follow-up duration beyond 2 years, highlighting the importance of regular long-term follow-up.

• 4422

### Intracameral bevacizumab as an adjunct to trabeculectomy: A one year prospective, randomized study

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(3) Ophthalmology, Rotterdam

**Purpose** To investigate the efficacy and safety of a single intracameral administration of bevacizumab to improve the outcome of trabeculectomy.

**Methods** Between April 2009 and November 2010, 144 consecutive, medically uncontrolled glaucoma patients who were scheduled for primary trabeculectomy were included in this prospective, randomized, double-blinded and placebo-controlled study. Patients were randomized to receive 50  $\mu$ l of either bevacizumab (1.25 mg) or placebo (balanced salt solution) in the anterior chamber peroperatively. Normal tension glaucoma patients also received mitomycin C application. Absolute success was defined as intraocular pressure (IOP)  $\leq$  18 mmHg and  $>$  5 mmHg with at least 30% reduction from baseline and no loss of light perception. The achievement of these criteria through the use of additional medical and/or surgical IOP-lowering treatments was defined as qualified success.

**Results** 138 patients completed a 12-month follow-up, 69 of whom had received a bevacizumab injection. IOP at 1 year postoperatively was significantly lower than baseline (placebo: 25.6 $\pm$ 9.9 vs. 11.9 $\pm$ 3.9, p<0.01; bevacizumab: 24.8 $\pm$ 8.1 vs. 11.9 $\pm$ 3.9, p<0.01), with no difference between bevacizumab- and placebo-treated patients (p=0.69). However, absolute success was higher in the bevacizumab group (71% vs. 51%, p=0.02), with the number of IOP-lowering interventions (needlings) being significantly lower in this group (13% vs. 36%, p=0.003). Complication rates were low and comparable in both groups.

**Conclusion** Peroperative administration of intracameral bevacizumab significantly reduces the need for additional interventions during the follow-up of patients undergoing trabeculectomy.

• 4423

### Bevacizumab together with MMC may have complementary effects in the improvement of surgical outcome after glaucoma filtration surgery

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(2) UZ Leuven, Ophthalmology, Leuven

(3) KU Leuven, Biology, Leuven

**Purpose** We first compared the effect of mitomycin-C (MMC) versus bevacizumab on the surgical outcome of glaucoma filtration surgery (GFS). Secondly, the complementary effects of MMC and anti-VEGF therapy were investigated.

**Methods** The effect of bevacizumab and MMC on surgical outcome was investigated in a mouse model of GFS. The 1st group received a subconjunctival (SC) injection of bevacizumab (1  $\mu$ l; 25  $\mu$ g); the 2nd group was treated during surgery with MMC (sponge soaked with MMC 0.02% for 2 minutes). Group 3 received a combination of MMC and a SC bevacizumab injection. Treatment outcome was studied by clinical investigation of bleb area and survival every other day. (Immu)stainings were performed to study angiogenesis (CD31) and fibrosis (Sirius Red) on postoperative day 14 and 30. VEGF-levels after MMC treatment were measured in rabbit aqueous humor (AH) samples by ELISA.

**Results** Bevacizumab and MMC were equally effective in improving surgical outcome. In both groups, bleb area was significantly improved with 53%, compared to control (NaCl). Blood vessel density and fibrosis were equally reduced, with 37% and 22%, respectively. Remarkably, one day after surgery, VEGF levels were significantly increased with 90% in the AH of MMC-treated eyes compared to control treated eyes. As compared to MMC monotherapy, combination of MMC and bevacizumab was able to increase bleb area with 18% by an additional reduction of angiogenesis and fibrosis, with 17% and 10%, respectively.

**Conclusion** Our data suggest that bevacizumab together with MMC may have complementary effects, due to upregulation of VEGF after MMC treatment.

• 4424

### What is the most optimal administration route of bevacizumab after glaucoma filtration surgery in mice?

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**Purpose** Glaucoma filtration surgery (GFS) frequently leads to surgical failure due to excessive postoperative wound healing. The current study was designed to investigate the effect on postoperative scarring of a single subconjunctival (SC), intracameral (IC) or intravitreal (IV) injection of bevacizumab.

**Methods** The effect of bevacizumab (AvastinTM, Genentech) was investigated in a model of GFS in C75BL/6 mice. Immediately after surgery, mice were divided in 3 groups (n=10 per group) and received a SC, IC or IV injection. In all groups, one eye was injected with bevacizumab (1  $\mu$ l; 25  $\mu$ g) and the other eye was used as a control and received an injection of NaCl (1  $\mu$ l; 0.9%). Treatment outcome was studied by clinical investigation of bleb area and bleb survival every other day. Mice were killed on postoperative day 14 and immunohistological analysis of angiogenesis and collagen deposition were performed.

**Results** In the mouse model of GFS, treatment using a SC, IC, IV injection of bevacizumab significantly improved surgical outcome by increasing bleb area with 53%; with 45% and with 49% respectively, compared to negative control. Bleb survival significantly improved only after SC injection with 37.5%. In all groups, blood vessel density was significantly reduced after bevacizumab treatment with 36%; with 31% and with 34%, respectively. All administration routes of bevacizumab significantly diminished collagen deposition with 27%, compared to negative control.

**Conclusion** This study shows that a subconjunctival injection of bevacizumab had the largest effect on surgical outcome compared to intracameral and intravitreal injection.

• 4425

**Refractive and axial length changes after trabeculectomy for open angle glaucoma**

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**Purpose** Predicting refractive and axial changes that might occur after glaucoma surgery should be taken into account when managing the patients that have had cataract surgery previously or at the time of trabeculectomy (combined procedures). The aim of our study is to provide data regarding the changes in refraction and axial length (AL) at one year follow-up after trabeculectomy for open angle glaucoma.

**Methods** This is a retrospective study assessing 48 patients that underwent trabeculectomy; 27 patients underwent cataract surgery at minimum 12 months after glaucoma surgery. Refractive status was analyzed using auto-refractometry and axial length was measured using non-contact biometry prior to the surgery and at one year follow-up. Primary outcomes were axial length, refractive status and intraocular pressure (IOP).

**Results** The difference between the mean final refraction and mean preoperative refraction in the study group ( $-0.68 \pm 1.05$  D vs  $-0.47 \pm 0.86$  D) was not statistically significant ( $P=0.215$ ). At one year follow-up, IOP was significantly reduced after trabeculectomy with a mean of  $8.95 \pm 2.93$  mmHg ( $p = 7.82 \times 10^{-13}$ ). AL was determined at six months and one year follow-up; the mean AL was significantly reduced at all time points (0.10 and 0.08 respectively;  $p$  value 0.015).

**Conclusion** The axial length reduction after trabeculectomy is small though still statistically significant. The decrease in axial length postoperatively was also correlated with the degree of IOP reduction. Following trabeculectomy the refractive status of the eye remained unchanged.

• 4426

**Risk factors for the development of glaucoma after vitreoretinal surgery**

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(2) Epidemiology, ErasmusMC, Rotterdam

**Purpose** To determine risk factors for the development of glaucoma after vitreoretinal surgery.

**Methods** Case-control study. Cases (n=96) were all patients who received a Baerveldt glaucoma implant after vitreoretinal surgery performed between 1991-2011. Controls (n=288) were a random subset of patients who underwent vitreoretinal surgery without subsequent glaucoma surgery. Cases and controls were matched with regard to the year in which the first vitreoretinal intervention was performed. The role of age, sex, number of interventions, type of intervention (plombe or vitrectomy), indication of first intervention, use of silicone oil, preoperative lens status, IOP and IOP lowering medication one week after the first intervention, history of glaucoma or OHT, and a positive family history of glaucoma were analyzed with logistic regression.

**Results** Cases were more often men ( $p=0.03$ ), younger at the moment of the first surgical procedure ( $p=0.005$ ), underwent more vitreoretinal procedures ( $p<0.001$ ), had on average a higher mean intraocular pressure ( $p=0.005$ ) and medication use ( $p=0.001$ ) 1 week after the first procedure and had more often a history of glaucoma or ocular hypertension ( $p<0.001$ ). The use of silicone oil became a significant risk factor after removing the number of interventions from the model ( $p=0.002$ ).

**Conclusion** Patients who develop glaucoma after vitreoretinal surgery have a different risk-profile compared to patients who do not develop glaucoma. This gives the opportunity to monitor certain subgroups.

• 4431

**Rigid gas-permeable contact lens correction of infant aphakia following congenital cataract surgery**

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(2) Ophthalmology Practice, Moers

(3) Contactlinsen Institut Haufser, Düsseldorf

(4) University Eye Hospital, Freiburg

This talk gives an overview of the actual data about the correction of infant aphakia with rigid gas-permeable contact lenses. The advantages and disadvantages of the use of rigid gas-permeable contact lenses after congenital cataract surgery will be discussed. Also, we will present our own data regarding the visual outcome of aphakic children treated with rigid gas-permeable contact lenses.

• 4432

**Stem cells in the central cornea: To be or not to be?**

MAJO F

Jules-Gonin Eye Hospital, Lausanne

Background: Since 1986, we believe that the cornea is epithelial stem cell free and all the epithelial stem cells are localized in the limbus. As a consequence, if the limbus and the corneal epithelial stem cell niche are destroyed the cornea loses its normal differentiation and transdifferentiate in conjunctiva. We call these diseases: limbal stem cell deficiency. According to the model of corneal epithelial stem cells we also classified diseases of the ocular surface as diseases related to a lack of epithelial stem cells. Discussion: Recent data have shown that there are epithelial stem cells in the central cornea of 5 different mammals. In human, central corneal epithelial cells can divide 50 times in vitro, and central corneal epithelial cells can survive for a long period after total destruction of the limbus. Furthermore, epithelial cells after epithelial ingrowths after LASIK or FEMTOLASIK exhibit corneal epithelial stem cells characteristics. In conclusion, all these data challenge the model we use to explain the renewing of the corneal epithelium and in consequence how we call and classify the diseases of the ocular surface.

• 4433

**The latest developments in the genetics of Fuchs corneal dystrophy**

BARATZ K

Rochester

Fuchs endothelial corneal dystrophy (FECD) is a common, familial trait. Several genes have been associated with the disease, including LOXHD1, SLC4A11, TCF4, ZEB-1 and COL8A2 which has been identified as a cause of an early-onset Fuchs-like disease. The evidence for these genetic associations will be reviewed and new data will be shared. To date, the most sensitive biomarker for FECD is an intronic TGC trinucleotide expansion in the TCF4 gene which is present in the majority of FECD cases and in a small proportion of normal control subjects. Within families, this trinucleotide expansion tends to segregate with the disease phenotype. The biology of trinucleotide expansion diseases and possible mechanisms by which TGC expansion may contribute to FECD will be presented. A brief discussion of recent advances in our understanding of the pathophysiology of FECD, such as the unfolded protein response and oxidative stress, will also be presented in order to highlight the challenges in linking the implicated causative genes with the biochemical pathways involved in the disease.

• 4434

**“No-touch” DMEK: Learning curve and outcomes**

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(3) First Department of Ophthalmology, Athens Medical School, Athens

Descemet Membrane Endothelial Keratoplasty (DMEK), as described by Gerrit Melles in 2002, is currently the most targeted technique of endothelial replacement, as only Descemet membrane and the endothelial monolayer are transplanted. Therefore, the postoperative anatomy of the cornea is not altered as in earlier techniques (DLEK, DSAEK) and visual rehabilitation is fast and frequently complete. In the present lecture, surgical videos of graft preparation and DMEK surgery with the standardized “no touch” techniques as described by Melles will be presented along with clinical outcomes with regards to corrected visual acuity, refraction, endothelial cell density, complications and secondary procedures.

**• 4435****Silica-collagen hybrid artificial cornea**

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Tissue-engineered artificial corneas could eventually replace cadaveric corneal transplantation, enabling sight-restoring procedures to many who do not have access to eye banking facilities. We investigated compositions of silica–collagen hybrid materials as potential artificial corneal substitutes. Hybrid materials were created from different proportions of collagen and silica precursors and manufactured to specific dimensions. Materials were implanted into animal corneas to determine if they supported epithelialization of their surface. The refractive indices of the hybrid materials ranged from 1.33 to 1.40 depending upon the composition and manufacturing characteristics. Transmissibility of 3:1 (silica:collagen ratio by weight) and 9:1 rehydrated xerogels was similar to that of normal rabbit corneas. Re-epithelialization of 5- to 6-mm-wide rabbit corneal epithelial defects was complete in  $5.5 \pm 2.4$  days ( $n = 6$ ), with evidence of epithelial stratification. Silica–collagen hybrid materials have properties that are promising for use as an artificial corneal substitute. Additional studies are required to determine long-term outcomes *in vivo*.

**• 4436****Modulators of corneal nerve regeneration**

BAZAN H

New Orleans

Damage to corneal nerves results in decreased sensitivity, dry eye, and neurotrophic keratitis. Rabbit corneas treated with pigment epithelial derived factor (PEDF) and the  $\omega$ -3 fatty acid docosahexaenoic acid (DHA) after lamellar keratectomy increase nerve regeneration and stimulates the synthesis of neuroprotectin D1 (NPD1). NPD1 also increases nerve density. The regenerated nerves contain similar proportion of calcitonin-gene related peptide (CGRP) to non-injured corneas. We compared the action of two smaller PEDF derivatives that maintain part of the bioactivity and can have better penetration. Animals after surgery were treated with a 44 mer-PEDF, with neuroprotective activity, and with a 34 mer-PEDF, with antiangiogenic properties, in association with DHA. The 44-mer PEDF plus DHA-treated group showed a two-fold increase in subepithelial corneal nerve area compared to the 34-mer PEDF+DHA and vehicle-treated groups. These results demonstrate that the use of NPD1 and 44-mer PEDF plus DHA could represent novel therapeutic approaches for managing eye conditions that perturb corneal nerve integrity. (Supported by NIH-NEI grant EY019465)

## • 4441

**C-Kit SCF receptor (CD117) expression and KIT gene mutation in conjunctival pigmented lesions**

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**Purpose** to investigate the presence of KIT gene mutations and immunoreactivity in 85 conjunctival melanocytic tumors and clarify the role of KIT as a potential therapeutic target in this group of patients.

**Methods** 85 conjunctival pigmented tumors (27 melanomas, 12 PAMs and 46 nevi) were immunostained for KIT. Intensity and pattern of expression were evaluated. Molecular analysis to identify KIT mutations was performed in 15 selected cases (tumor-rich areas >50%). KIT immunostaining score and pattern were statistically related to patients' age, sex, diagnostic category, presence of relapse, disease-free survival, presence of metastases, metastasis-free survival, limbal versus non-limbal tumor location and thickness of melanomas.

**Results** KIT stains was documented in 48% of melanomas, 50% of PAMs and 24% of nevi. The mean score of KIT staining in the melanomas/PAMs group was significantly different from nevi ( $p=0.0076$ ). No statistically significant differences were detected between either c-kit immunostaining score or pattern and each of the other clinicopathologic parameters considered. No KIT gene mutations were detected in melanomas and nevi. A silent mutation/polymorphism in KIT exon 13 was found in one PAM.

**Conclusion** Despite the high level of KIT immunostains in conjunctival PAM and melanoma, this parameter seems not to be a good predictor for the presence of molecular mutations. KIT-activating mutations should be considered an uncommon event in these tumors.

## • 4442

**Circulating tumour DNA (ctDNA) in metastatic uveal melanoma (MUM): Correlation with outcome in 87 patients (pts) from Institut Curie**

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**Purpose** CtDNA can be detected in the plasma of MUM pts using a real-time PCR based on the pyrophosphorylation-activated polymerization (bi-PAP) targeting the most frequent GNAQ and GNA11 mutations present in 85% of UM (Madic et al, 2012).

**Methods** May 2011-March 2013:87 MUM pts were included in 3 prospective studies to assess the bi-PAP assay and analyse clinical and outcome correlations.

**Results** Median (med) age 57, primary tumor med diameter 15 mm; enucleation 32, proton beam 52, iodine disk 3. Enucleated eyes showed mostly mixed histotype and genomic high risk by array-CGH: 8q gain and/or 3p loss. With a med disease free interval of 39 months (mo), 83 pts developed liver metastases first, with radiological miliary disease in 55; 4 had extra hepatic lesions without liver involvement. 35 pts were enrolled at the time of metastasis diagnostic. With a med follow-up of 8 mo, 28 patients had disease progression and 34 died of metastasis. The med survival was 13 mo. Tumor samples were available in 82 pts, genotyping is ongoing for 8. GNAQ 626A>T or A>C, GNA11 626A>T and rare mutations were found in 9, 19, 26 is and 6 cases respectively; 14 were wild-type tumors. CtDNA was detected in 51/54 samples (med 30 copies/ml, range 1-11421) and correlated with the metastatic tumor burden as assessed by liver MRI (med 64 cm<sup>3</sup>, range 0.2-7384). Correlation with progression-free and overall survival will be presented.

**Conclusion** ctDNA is a promising tool to assess the tumor burden and the micrometastatic dissemination of uveal melanoma, and a potential biomarker to evaluate the efficacy of targeted therapies in pts carrying GNA Q/11 mutated tumors.

## • 4443

**Uveal melanoma among Finnish octogenarians**

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**Purpose** To report clinical characteristics of uveal melanoma (UM) among Finnish octogenarians.

**Methods** Population-based study of 86 patients 80 years of age or older, treated in 1997-2011 at the Helsinki University Central Hospital for UM. Patients had a minimum of 2 years follow-up if still alive.

**Results** Of the 86 patients, 36 (42%) were female and 50 (58%) were male. Median age was 82.9 years (range, 80-93), 5 had a previous nevus, median tumor height was 4.8mm (range, 1.1-17.9), median largest basal diameter was 12.7 (range, 4.3-22). Following the 7th edition of TNM tumor classification, 33% were T1, 19% T2, 22% T3, and 26% T4, and 28% were stage I, 32% stage II, 33% stage III, and 6% were stage IV (i.e. metastases at diagnosis). 70 patients were treated with brachytherapy (iodine in 38 and ruthenium in 32), 12 underwent primary enucleation, 1 eye was exenterated and 2 small tumours were observed. Median visual acuity was 0.2 (interquartile range, 0.6 to counting fingers) and in the fellow eye 0.7. At last follow-up, 20 patients had died of metastasis, 21 of other causes, 3 of a second cancer (prostate, renal, urinary bladder), death certificate was not yet reviewed in 16, and 26 were alive a median of 5.1 years after treatment (range, 1.4-11.1). The 5-year Kaplan-Meier survival was 75% overall, and 93% for stage I, 79% for stage II, and 62% stage III; all patients of stage IV died within 1.3 years. The corresponding all-cause mortalities were 66%, 55%, and 29%.

**Conclusion** Taking competing causes of death into account, less than 25% of octogenarians die of uveal melanoma within 5 years.

## • 4444

**Family relationships of recent familial uveal melanomas in Finland**

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**Purpose** Attention has recently focused on hereditary predisposition in uveal melanoma. We describe relationships between patients with familial uveal melanomas prospectively diagnosed in Finland during the last five years.

**Methods** Prospective ascertainment of uveal melanoma patients with a positive family history between 2008 and 2012 in a national ocular oncology center managing essentially all uveal melanomas in Finland, a high risk region for developing this rare cancer.

**Results** Of 311 new patients with uveal melanoma, 8 (2.6%; 95% confidence interval, 1.1-5.0) eventually gave a positive family history upon specific questioning and encouragement to query among relatives. The index cases were 40-83 years old when the diagnosis was made (mean, 61) and 2 of them came from the same family. The previous cases in these families had been 26-82 years old at the time of diagnosis, which had been made 3-21 years earlier. The earlier patients had the following relationship to the index case: 1 son, 1 father, 1 mother, 1 grandmother, 2 aunts, 1 uncle, and 1 cousin. Of them, 5 had died of uveal melanoma and 2 were alive. On the other hand, none of the index patients have so far developed metastases or died.

**Conclusion** Mutations of BAP1 so far have explained some but not all familial cases of uveal melanoma. Identification and further analysis of familial uveal melanomas will help to understand the emerging genetic diversity of uveal melanoma.

## Joint Meeting: OOG : Melanocytic lesions

### • 4445

#### Quantitative proteomic analysis of uveal melanoma reveals potential therapeutic targets

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**Purpose** To identify potential therapeutic targets in uveal melanoma by global proteomic analysis of tumours with high- (HR) or low- (LR) risk of developing metastatic disease.

**Methods** Proteins were extracted from fresh-frozen tissues from 10 HR and 10 LR UM and from 22 normal choroid samples, subjected to isobaric tagging for relative quantification (iTRAQ) and analysed by nanoLC-MS/MS. Peptide identification was performed using Protein Pilot with a False Discovery Rate set at 5%. Differential expression of proteins and their relative abundance between the groups was investigated by supervised and unsupervised hierarchical clustering and outlier analyses.

**Results** We identified 2651 unique proteins. Compared to normal choroid, the tumours showed 672 proteins up-regulated more than 1.5-fold and 459 down-regulated more than 1.5-fold. Of these, 18 proteins with known functions in tumour development/progression were identified and shown to be differentially expressed between HR and LR samples. Four of these have been further validated by immunohistochemistry in a larger cohort of patient samples.

**Conclusion** Using quantitative proteomic analysis and immunohistochemistry we have identified novel potential therapeutic targets in UM. One of these in particular is a tumour suppressor associated with chemosensitivity in other cancers, but not yet described in UM. This opens a novel therapeutic avenue that is being further investigated.

### • 4446

#### Uveal metastases from carcinoid tumours: Clinical and therapeutic assessment

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**Purpose** To define the specific clinical characteristics of uveal metastases from carcinoids and the minimal invasive therapeutic approach.

**Methods** Four consecutive cases of symptomatic uveal metastases from carcinoid tumors were included in this prospective analysis. Each patient underwent full ophthalmological examination, including A-B scan ultrasonography (US), fluorescein (FA) and indocyanine green angiography (IGA), and optical coherence tomography (OCT) at baseline and during follow-up. Follow-up examination was performed at 1 month and every 3 months thereafter.

**Results** Mean follow-up was  $17 \pm 6$  months. Three patients were affected by choroidal metastases (75%) whereas one patients by iris metastasis (25%). Choroidal metastases were ipo-pigmented, mainly located at the posterior pole and characterized by medium internal reflectivity at US, prominent vascular net at ICG and perilesional serous retinal detachment at OCT. Each patient was treated by photodynamic therapy (PDT) with verteporfin as sole focal treatment. Mean PDT sessions were 1.6 (1-3 sessions). Two patients (50%) showed clinical regression of the treated lesions, whereas in the others two patients (50%) tumor size remained stable during follow-up. Subretinal fluid resolved in all choroidal lesions. BCVA increased in three patients (75%) and remained unchanged in one patient (25%).

**Conclusion** Uveal metastases from carcinoids are characteristically ipo-pigmented and highly vascularized. Photodynamic therapy appears to be an effective, safe and well-tolerated treatment option for the management these metastases.

### • 4447

#### Use of dexamethasone 0.7mg intravitreal implant in radiation macular edema

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**Purpose** To evaluate the efficacy and safety over one year of follow-up of dexamethasone 0.7mg intravitreal implant in patients with radiation macular edema (ME) after proton beam therapy for choroidal melanoma

**Methods** Five patients' charts were retrospectively reviewed.

**Results** All patients received a radiation dose of 60 cobalt Gray equivalent. Radiation ME occurred within a mean time of 26 months after irradiation. Mean preinjection VA was 41 ETDRS letters. Two months after injection, mean VA was 47 ETDRS letters. It improved for 3 patients (+4; +9 and +15 letters) and remained unchanged for 2. Mean CRT was 331  $\mu$ m. Over one year of follow-up, two patients underwent 2 injections of dexamethasone performed 5 months after the first injection. The gain of VA was +8 and +23 letters with a decrease in CRT of 158 and 262  $\mu$ m respectively. Intraocular pressure increased for 1 patient. One patient was enucleated due to a local recurrence of his choroidal melanoma. Another patient presented with a radiation neuropathy with a great loss of visual acuity.

**Conclusion** Intravitreal dexamethasone implant can improve VA in radiation ME. Its beneficial effect lasted up to 5 months. Other causes of vision loss may jeopardize this beneficial effect.

• 4451

**Comparison of the biometric measurements obtained using IOL Master and ALADDIN systems**

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**Purpose** To compare anterior segment measurements of two optical systems of patients with clear lens and cataract

**Methods** 34 cataractous eyes and 32 eyes with clear lens were examined using the Zeiss IOL-Master and Topcon Aladdin system. In all eyes we measured Axial Length (AL), Keratometry (K1, K2) and Anterior chamber depth (ACD). The results were compared and analysed using Bland-Altman plots

**Results** Comparison of the eye lengths, as well as of the keratometric and ACD measurements showed good correlation and agreement between the measurements obtained by both methods. Standard Deviation (SD) for Axial Length difference is about 0,034 for clear lens eyes and 0,028 for cataractous eyes. SD for K1 and K2 difference is for clear lens eyes 0,23/0,29 and 0,17/0,18 for cataractous eyes. ACD differences are respectively 0,11 and 0,15

**Conclusion** The comparisons between IOL Master and the ALADDIN show a very good alignment for Axial Length for both groups of patients. There was also acceptable alignment for K1, K2 and ACD measurement. Both the analyzed instruments show a good correlation and precision

• 4452

**Harmlessness of 1 mg cefuroxime intracameral injection at the end of cataract surgery**

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**Purpose** Intracameral 1mg cefuroxime injection at the end of cataract surgery is included in the good practice in France and a lot of countries. Few studies have shown a macular toxicity for high doses of cefuroxime in particular in a case of dilution error for example.

**Methods** We propose a study on 18, of simple cataract operated, eyes : by phakomulsification, the same surgeon, without particular surgical complications, and no posterior capsule rupture. Only patients with medium cataract and a normal macula were included in the study. A time-domain macular OCT was pre-operatively made, and then at 7 and 30 post-operative days. The principal criterion of judgement was the central macular thickness at 30 post-operative days.

**Results** The average pre-operative central macular thickness was 209,11 +/- 29,44 microns (143-279), at 7 post-operative days the average was 214,44 +/- 28,42 microns (145-267), and at 30 post-operative days was 219,44 +/- 27,41 microns (145-253). The differences observed between the pre and post-operative values were not significant.

**Conclusion** These results show that at a dose of 1 mg, as an intracameral injection, and without posterior capsular rupture, cefuroxime does not significantly increase the central macular thickness. A study with a larger pool of patients would certainly confirm what the actual clinical practice lets us foresee.

• 4453

**Intravitreal corticosteroids as first-line adjunctive treatment in acute post cataract surgery endophthalmitis**

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**Purpose** To assess the effect of intravitreal corticosteroids as first-line adjunctive treatment in acute post cataract surgery endophthalmitis.

**Methods** We conducted a multicenter prospective, randomized, single-masked study including patients with acute post cataract surgery endophthalmitis. All patients received the initial intravitreal antibiotics (vancomycin 1mg, ceftazidime 2mg) with intravitreal betamethasone (400 µg) in the group 1 but not in the group 2. At day 2, all patients had the same intravitreal antibiotics with betamethasone (400 µg). A prompt or deferred vitrectomy could be associated with this treatment if baseline visual acuity was limited to light perception. The primary endpoint was the BCVA (logMAR) and the secondary endpoint was the impact on intraocular pressure

**Results** We report the preliminary results on the first 54 patients; 24 patients were included in the group 1 and 30 in the group 2. The BCVA was better in the group 1 at 8 days ( $1.44 \pm 0.90$  vs  $1.90 \pm 1.0$  logMAR), at one month ( $0.50 \pm 0.69$  vs  $1.24 \pm 1.11$  logMAR) and at 3 months ( $0.52 \pm 0.87$  vs  $0.99 \pm 1.13$  logMAR). The confidence intervals were overlapping, except at one month, suggesting a statistical significance at this point. There was no difference in both groups considering the intraocular pressure at day 8: 14.13 mmHg (IC 95%: 10.29-17.97) in the group 1 and 12.52 mmHg (IC 95%: 10.49-14.55) in the group 2.

**Conclusion** Intravitreal corticosteroids as a first line adjunctive treatment in acute post cataract surgery endophthalmitis seem to accelerate the recovery of visual acuity but do not seem to have an impact on the final functional outcome. These results need to be confirmed by the statistical analysis of the cohort of 100 cases.

• 4454

**Optimizing number of postoperative visits after cataract surgery – a safety perspective**

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**Purpose** To evaluate safety perspectives, when the standard routine after cataract surgery is no planned postoperative visit.

**Methods** A prospective, observational cohort study was performed on all cases of cataract surgery during a 1-year period, at one institution (n=1249). The study group was all patients following the standard procedure of the clinic. As a control group all patients having surgery during one month (March) was chosen. All these patients had a planned postoperative visit. The standard routine is no planned postoperative visit. A postoperative visit is planned if there are other significant eye diseases, for instance glaucoma, wet AMD and diabetic retinopathy. Outcome measures were; any planned postoperative visit, any complications and/or adverse events, postoperative visual acuity and any postoperative control/contact initiated by the patient.

**Results** There were no differences in age, sex, complications/adverse events and postoperative visual acuity between the study group and control group. 9 percent of the patients initiated a postoperative contact. 26% of the patients who initiated postoperative contact had also a scheduled visit. Reasons for unplanned contacts were visual disturbance, redness and/or chafing, pain and anxiety. Most unplanned contacts (68%) initiated by the patients were first eye surgery.

**Conclusion** It is possible refraining from planned postoperative visits in cases of uncomplicated cataract surgery. Before surgery patients with comorbidities need individual planning of their postoperative care. Preoperative information is important and the clinic should have resources to answer questions from patients and be prepared for the need of unplanned additional postoperative visits.

• 4455 / S067

**Mydriaser pupillary dilation for cataract surgery:  
A clinical and economic study**

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**Purpose** Mydriaser<sup>®</sup> is an ophthalmic insert containing phenylephrine and tropicamide. A prospective clinical evaluation of Mydriaser for pupillary dilation prior to cataract surgery was conducted. The impact on nurse time of using Mydriaser compared to eye drops was also evaluated.

**Methods** The following data were collected prospectively: amount of time spent inserting Mydriaser, patient satisfaction score (on a scale of 1-10), pupil size (on a pre-defined categorical scale of 1 (small pupil) to 4 (very large pupil) and any adverse events related to the insert. An economic model of mydriasis was also developed.

**Results** Mean nursing time spent inserting Mydriaser was 1.52 minutes (SD=0.6, n=85), mean patient satisfaction score was 8.97 (SD=2.32, n=97) and mean pupil size was 3.32 (SD=0.66, n=114) corresponding to a large to very large pupil size. The insert had extruded unnoticed in 3 cases. For a national population (England), the estimated nurse time saved was 1,159 hours per week when using the single application Mydriaser compared to repeated instillation of eye drops to achieve mydriasis.

**Conclusion** Mydriaser is a safe and efficacious alternative to topical drops for pupillary dilatation. The rate of insert extrusion was low compared to that published in a smaller study reporting on a much smaller sample. This may reflect a learning curve for Mydriaser insertion. The economic analysis demonstrated that using Mydriaser could potentially free up nursing time to spend on other responsibilities.

**Commercial interest**

• 4456 / S068

**Cataract surgery and retinal vein occlusion:  
Is there an association?**

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**Purpose** To describe and characterise patients who were noted to have developed postoperative retinal vein occlusion (RVO) soon after cataract surgery and to hypothesise the underlying mechanism of such manifestation

**Methods** All the patients who developed post-operative retinal vein occlusion (RVO) soon after cataract surgery between April 2012 and April 2013 in Cumberland Infirmary, Carlisle, United Kingdom, were consecutively recruited. All cataract surgeries were performed under topical anaesthesia. All the patients were assessed pre-operatively and 4 weeks after the cataract surgery.

**Results** A total of 3 patients were included in this study. All 3 patients were male and mean age of the cohort was  $82 \pm 10$  years. The mean preoperative best-corrected visual acuity (BCVA) of the cohort was 78.3 ETDRS letters. Vascular risk factors were identified in all patients, including previous RVO (66%), hypertension (100%), diabetes (33%) ischemic heart disease (33%) and smoking (66%). All patients underwent phacoemulsification with IOL implantation without any complication. Time interval between surgery and diagnosis of RVO was  $73.3 \pm 39.7$  days. Postoperative BCVA of the cohort was 50 ETDRS letters. Two patients required 0.7mg intravitreal dexamethasone implant (Ozurdex).

**Conclusion** Vascular risk factors were identified in all the patients who were noted to develop RVO following cataract surgery. We hypothesise that the fluctuation of intraocular pressure during cataract surgery predisposes to the development of intraoperative retinal vein occlusion in our cohort. Preoperative counselling regarding the risk of RVO before the cataract surgery may be indicated.

• 4457 / S073

**Morphological and proliferative studies on ex vivo cultured  
human anterior capsule lens epithelial cells**

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**Purpose** The proliferation and migration of lens epithelial cells (LECs) that remain in the capsular bag after cataract surgery can lead to development of posterior capsule opacification (PCO) - a major cause of post-operative visual loss. The multipotency of anterior lens capsule (LC) LECs is still controversial. We aimed to test the proliferating and migrating potential and to characterize structurally the ex vivo cultured LECs growing out of human anterior LC.

**Methods** The explants of LCs were obtained from uneventful cataract surgery and consisted of basal membrane and monolayer of LECs that were cultivated under adherent conditions. The size and shape of the outgrowing cells were recorded by scanning electron microscopy (SEM) and their migration and proliferation potential were followed on a daily basis throughout their continued growth using light microscope.

**Results** The migration and proliferation of anterior LC LECs filling up the denuded region of the LC could be followed by light microscopy. These cells could also migrate to the opposite side of the capsule as shown by both, light microscopy and SEM. The migration and proliferation of LECs on glass or plastic culture surfaces could be shown by both techniques, while the distribution of the LECs and their morphology could be analyzed in details by SEM.

**Conclusion** Classic light microscopy and SEM studies can be used to show that human anterior LC harbors LECs that can migrate and proliferate, suggesting their multipotency and putative stem cell nature. The anterior LC explants can be used to study PCO and the potential of different pharmaceutical or physical treatments against PCO development.

• 4458 / S083

**Halo size after implantation of a multifocal intraocular lens**

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**Purpose** To determine the size of a halo in the visual field induced by bright light after cataract surgery and implantation of an aspheric apodized diffractive multifocal intraocular lens (IOL) or aspheric monofocal IOL.

**Methods** This prospective observational study comprised 54 healthy eyes (mean age  $72.6 \pm 10.4$ ) of 54 subjects having cataract surgery with bilateral implantation of an AcrySof ReSTOR SN6AD1 IOL (32 eyes, multifocal group) or AcrySof SN60WF IOL (22 eyes, monofocal group). Six months postoperatively, halo size was measured using the Vision monitor. Optotypes of low luminance ( $5\text{cd}/\text{m}^2$ ) were presented at a distance of 2.5 m. The visual angle subtended by the radius of the halo was calculated in minutes of arc (arc min). Best-corrected distance logMAR visual acuity (VA) was evaluated with high-contrast (HC-96%) and low-contrast (LC-10%) logMAR Bailey-Lovie letter charts.

**Results** The mean radius of the halo was  $219.3 \pm 42.1$  and  $194.0 \pm 55.3$  arc min ( $p > 0.05$ ) in the multifocal and the monofocal groups respectively. The HC and LC VA means showed significant differences between the two groups, being worse in the multifocal group. Also, the HC and LC-VA exhibited a significant correlation with halo size ( $r = 0.52$ ,  $r = 0.57$  respectively).

**Conclusion** Mean halo size was not significantly different between the multifocal ReSTOR SN6AD1 and the monofocal AcrySof SN60WF IOLs. Worse HC and LC-VA values were related to higher halo size measurements in the IOLs groups.

• 4461

**Ranibizumab versus bevacizumab for neovascular age-related macular degeneration: Results from the GEFAL noninferiority randomized trial**

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**Purpose** To evaluate the relative efficacy and safety profile of bevacizumab versus ranibizumab intravitreal injections for treatment of neovascular age-related macular degeneration (AMD)

**Methods** Multicenter, prospective, non-inferiority, double-masked, randomized clinical trial, performed in 38 French ophthalmology centers. Patients were randomly assigned to intravitreal administration of bevacizumab (1.25 mg) or ranibizumab (0.50 mg). Patients were followed for one year, with a loading dose of three monthly intravitreal injections, followed by an as-needed regimen (one injection in case of active disease) for the remaining nine months with monthly follow-up.

**Results** Between June 2009 and November 2011, 501 patients were randomized. In the per protocol analysis, bevacizumab was non-inferior to ranibizumab (bevacizumab minus ranibizumab +1.89 letters, 95% confidence interval -1.16 to +4.93, p<0.0001). The intention-to-treat analysis was concordant. The mean numbers of injections were 6.8 in the bevacizumab group and 6.5 in the ranibizumab group (p=0.39). Both drugs reduced the central subfield macular thickness, with a mean decrease of 95 µm for bevacizumab and 107 µm for ranibizumab (p=0.27). There were no significant differences in anatomical secondary criteria. The proportion of patients with serious adverse events was 12.6% in the bevacizumab group and 12.1% in the ranibizumab group (p=0.88). The proportion of patients with serious systemic or ocular adverse events was similar in both groups.

**Conclusion** Bevacizumab was non-inferior to ranibizumab for visual acuity at one year with similar safety profiles. The results are similar to previous head-to-head studies.

• 4463

**Non-invasive discrimination between perfused and occluded vessels by optical coherence tomography**

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**Purpose** To noninvasively distinguish between perfused and occluded vessels from OCT scans of the human retina.

**Methods** Two specific signatures of the vascular tree of the human retina do exist, the hyper-reflectivity from the top of the vessel, and the shadow due to light absorption by haemoglobin. This shadow effect is notorious on the retinal pigment epithelium (RPE) being responsible for the dark appearance of the perfused vascular network on OCT fundus images. While the hyper-reflectivity (due to the vessel) is always present, the shadow effect on the RPE only exists for perfused vessels. Thus, this method allows the discrimination between perfused and occluded vessels by a non-invasive OCT scan of the human retina.

**Results** The haemoglobin on perfused vessels act as a natural dye on optical coherence tomography data. By computing the integral of OCT A-scan values over specific layers, it was demonstrated the possibility to discriminate between perfused and occluded vessels of the human retina noninvasively. In addition, the extent of the perfused vascular network possible to identify on OCT fundus reference is similar to that of colour fundus photography, and notably allows the identification of the non-perfused vascular network which colour fundus photography do not.

**Conclusion** The presented method compares favourably to colour fundus photography in discriminating between normal and occluded vascular tree, within the same scan, and provides functional information noninvasively.

• 4462

**Fast fully-automated multimodal image co-registration (optical coherence tomography, colour fundus photography, red-free, fluorescein angiography)**

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**Purpose** To automatically co-register optical coherence tomography (OCT), colour fundus photography (CFP), red-free (RF) and fluorescein angiography (FA).

**Methods** The burden of manually assisted co-registration and the number of images taken per day prevent its widespread use. An algorithm able to co-register images in between the major modalities in due time is herewith proposed. Typically, OCT fundus references translate into a poorly detailed vascular network, thus rendering difficult its co-registration to other imaging modalities. A recently developed method (by our group) to compute the vascular network from OCT to the level of detail of CFP was used. A set of vessel features is computed and an iterative process estimates the transformation required to co-register these locations. At each step the number of inliers is determined and the process repeats. Images of 20 eyes from 13 patients that underwent high-definition OCT, CFP and FA, were co-registered. These images were manually segmented by a grader. Two additional sets were co-registered and evaluated by an expert: 102 OCT/CFP image pairs from 51 subjects, and 40 FA/RF image pairs from 20 patients.

**Results** A skeleton overlap metric was defined and computed based on the vessels skeleton (0–no overlap, 1–full overlap) for the first set. An average overlap of 0.93±0.03 (N=60) is reported. From the expert evaluation, 91% of images were successfully co-registered. For the registration of a given pair, the algorithm takes 1.68±0.38 seconds on an Intel Core i7-2600k, 3.4 GHz computer.

**Conclusion** The achieved level of co-registration, render this process an asset to the clinical daily practice and research.

• 4464

**Enhanced 3D retinal vascular network reconstruction from high-definition SD-OCT**

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- (2) *IBILI - Institute for Biomedical Imaging and Life Sciences, FMUC - Faculty of Medicine, University of Coimbra, Coimbra*

- (3) *Mathematics Section, Department of Science and Technology, Open University, Lisboa*

**Purpose** To compute the 3D vascular network of the human retina from high-definition spectral-domain optical coherence tomography (OCT).

**Methods** A method able to compute a fundus reference from SD-OCT scans of the human macula to an extent similar to what can be achieved with color fundus photography was recently proposed by our research group. We resort to this new method and to an automatic supervised-learning classification algorithm (both submitted for publication) to identify OCT A-scans that cross the vascular network. A multi-scale wavelet analysis is performed on each of these A-scans to identify the transition between the hyper-reflectivity due to the vessel and the shadow cast due to light absorption by hemoglobin. This transition is set as the vessel depth location, and the respective diameter estimated as the width from the computed fundus reference.

**Results** Eyes of healthy controls (N = 10) and eyes (ETDRS levels 10 to 35) from type 2 diabetic patients (N = 20) were imaged with the Cirrus HD-OCT (Carl Zeiss Meditec, Dublin, CA, USA) and photographs were obtained with the Topcon TRC50DX (Topcon Medical Systems, Inc., Oakland, NJ, USA) or the Zeiss FF 450. The vascular tree from the OCT reference presents an extension similar to that of the color fundus photography, 99.7(10.68)% (average(SD)). The 3D reconstruction of the vascular network allows to clearly distinguish between crossovers and bifurcations and the vascular network distribution is in agreement with the known anatomy of the human retina.

**Conclusion** The herewith presented method allows the visualization of the vascular network in 3D and compares favourably to the previous method both in terms of quality and extension of the vascular tree.

• 4465

**En face 3D-SDOCT images and the saltmarshes sign**

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(2) *Retina, Belgrade*

**Purpose** To investigate the signal intensity obtained from the vitreoretinal interface using the spectral domain 3D OCT images.

**Methods** Review of spectral domain optical coherence tomography (OCT) examinations; mapping of the intensity of the tomographic signal over the posterior pole using the 3D reconstruction software; Retrospective study of the signs revealed. Chi-square was used.

**Results** Mapping the signal intensity over the posterior pole was named as Vitreoretinal Interface Signal Topography (V.I.S.I.T); VISIT images offer views of the epicenters and retinal folds associated with epiretinal membranes, a tool to followup the progress of the disease and to investigate the direction of the tractional forces; VISIT images allow the visualization of the borders of internal limiting membrane peeling and the inner retinal defects after an ILM rhexis; VISIT images revealed white plaques at the vitreoretinal interface, which were named as saltmarshes (SAM); SAMs are reproducible, exhibit interocular symmetry, faint in older ages and they correlate with the epicenters of ERMs.

**Conclusion** En face VISIT images offers unique information about the vitreoretinal interface; the saltmarshes sign may indicate the areas of strong vitreoretinal adherence and strain and/or the cortex remnants after a posterior vitreous detachment.

• 4466

**Visualization of 3D retinal microcapillary network using OCT**

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**Purpose** To present a novel method for noninvasive visualization of 3D retinal microcapillary network (RMN) using optical coherence tomography (OCT) reflectivity maps and to validate its clinical usefulness in vascular retinal diseases.

**Methods** OCT imaging was applied to 43 patients with diabetic retinopathy, CRVO, BRVO and in 13 healthy subjects. OCT maps of RMN from different retinal layers were reconstructed and compared with fluorescein angiography.

**Results** OCT showed the RMN of 20 microns in diameter, revealed vascular nonperfusion and identified microexudates that were not visible on clinical examination and fundus photography.

**Conclusion** OCT is capable to precisely detect the RMN and can be used for noninvasive diagnosis and monitoring of early stages of retinal vascular diseases.

• 4467 / F058

**Swept source optical coherence tomographic findings in a case of bilateral macular coloboma**

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(2) *Singapore National Eye Centre, Singapore*

**Purpose** To describe the findings of macular coloboma using swept-source optical coherence tomography (SS-OCT)

**Methods** Case report

**Results** A 35-year-old man was found to have bilateral deep excavation of the maculae and was diagnosed clinically with bilateral macular coloboma. SS-OCT showed deep excavation of the sclera corresponding to the area of coloboma. There is loss of overlying retinal pigment epithelium (RPE), neurosensory retina and choroid within the area of excavation. A thin membranous structure is seen overlying the area bared sclera. At both edges of the coloboma, intraretinal cystic changes can be seen.

**Conclusion** The SS-OCT is able to image the posterior border of the deep coloboma in this case. In addition, the detailed structure of the retinal layers can be studied. This membranous structure likely represents the remnants of degenerative neurosensory retina and internal limiting membrane. In addition, we also demonstrated intraretinal cysts, not previously reported, at the edges of the coloboma. The use of SS-OCT technology has enabled the capturing of the retina, RPE and choroid at a much better resolution and this has served to provide greater understanding of the anatomical changes seen in macular coloboma.

• 4468 / F039

**Wide field imaging in Coat's disease**

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*Ophthalmologie, Reims*

**Purpose** To report the usefulness of wide field imaging and angiography in patients with Coat's disease.

**Methods** In 5 consecutive patients, wide field imaging using scanning laser ophthalmoscopy and angiography (Optomap) was performed before treatment. The laser was performed on the basis of the wide field images. When the ophthalmologist considered that the treatment was complete, the imaging was repeated. After reviewing the images, the ophthalmologist decided whether further treatment was required.

**Results** Among all patients, wide field imaging enabled to monitor the localization of laser treatment with respect to the vascular abnormalities and non perfusion areas displayed on angiography. In all cases, additional laser treatment was performed on the basis of image lecture although the treatment had been considered to be complete by the ophthalmologist.

**Conclusion** Wide field imaging and wide field angiography enables to target laser treatment in Coat's disease.

## • 4471

**Early and sustained treatment modifies the phenotype of birdshot retinochoroiditis**

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(3) University Hospital Zurich, Department of Ophthalmology, Zurich

(4) University of Lausanne, Lausanne

The present appraisal of Birdshot retinochoroiditis (BCR) is based on obsolete criteria that only represent advanced disease. Early diagnosis of BCR can only be done using indocyanine green angiography (ICGA) showing the typical choroidal involvement. The aim of this study was to investigate long-term follow ups of BRC patients regarding the impact of early treatment on the phenotype of BRC, both on ICGA findings as well as fundus appearance. 13 patients had sufficient data to be included in the study. Out of 13 patients, 8 showed typical birdshot lesions at presentation according to the presently used criteria. A significant proportion of the "no lesions" group did not develop typical birdshot lesions throughout the course. Mean treatment delay was  $45.50 \pm 33.39$  months in the "typical lesions" group and  $5.80 \pm 2.95$  months in the "no lesions" group. Early treatment can avoid the apparition of typical BRC lesions in the same fashion as early and heavy treatment can avoid the development of "sunset glow" fundus in Vogt-Koyanagi-Harada disease (VKh). Early diagnosis relies heavily on ICGA, that together with FA, visual field testing and presence of HLA-A29 antigen allows the diagnosis before typical birdshot lesions can be seen.

## • 4472

**Combined approach of inflammatory choroidal neovascularization indicates improved outcome**NERI P, PIRANI V, ARAPI I, CAPUANO V, GIOVANNINI A, MARIOTTI C  
The Eye Clinic, Polytechnic University of Marche, Ancona

Inflammatory Choroidal Neovascularization (CNV) can be one of the most severe sequelae in patients with uveitis. The outcome of subfoveal inflammatory CNV is poor if untreated: several procedures have been considered, even though there is lack of guidelines. On the other hand, the better knowledge of CNV pathophysiology may suggest a suitable treatment strategy. The combination of steroids and immunosuppression represents an important aspect of inflammatory CNV treatment. This ensures suitable control of inflammation as well as the reduction concomitant steroids dose. Nevertheless there are cases which do not show a fully satisfactory response. Recently, the role of intravitreal anti-Vascular Endothelial Growth Factor (VEGF) has become primary in the treatment of neovascularizations. At this time, the combination of anti-VEGF drugs and immunosuppressives seems to be the best option for the management of inflammatory CNV. In summary, CNV secondary to uveitis is a severe sequela, which can lead to significant visual impairment. Although no guideline is provided, the current medical literature can give the basis for a successful treatment strategy, suggesting that combination of immunosuppresives and anti-VEGF is recommended.

## • 4473

**Better monitoring of intraocular inflammation by laser flare photometry and diversified therapeutical approach could mean improved outcome for JIA**

BODAGHI B

Paris

Slit lamp biomicroscopy is widely used to evaluate the importance of anterior segment flare and cells in children with anterior uveitis. However, different studies have clearly shown that other tools such as laser flare photometry may improve the monitoring during the follow-up. Laser flare photometry showed for the first time that active ocular inflammation may be associated with a significant level of flare even in the absence of detectable cells. This is a major finding to start a therapeutic approach or change it for a more aggressive strategy and monitor the decrease of flare. Moreover, the level of flare decrease under therapy may predict further serious complications such as secondary glaucoma or cataract. Based on our modern monitoring tools, therapeutic strategies have been progressively modified, allowing a better control of chronic uveitis. Efficacy and tolerance of anti-TNF alpha agents will be discussed and alternatives in severe and resistant cases will be discussed.

## • 4474

**The use of laser flare photometry to assess therapeutical approaches for uveitis**

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(2) Department of Ophthalmology, St. Franziskus Hospital, Muenster

Laser flare (LF) photometry is a standardized and reproducible method for grading the breakdown of the blood-aqueous-barrier by in-vivo measurements of the protein content in the anterior chamber. Whereas anterior chamber cell-grade directly correlates with the activity of anterior uveitis, high LF values may not only be observed during active inflammation but also as a remnant of chronic uveitis with irreversible damage of the blood-aqueous barrier. The talk provides an overview of published and own data, analyzing the impact of LF photometry as a decision-making tool for timing ocular surgery and selection of surgery techniques (e.g., lensectomy vs. phacoemulsification with primary IOL implantation; filtrating vs. non-filtrating vs. drainage-implants vs. cyclodestructive surgery). Furthermore, the role of LF in the context of secondary complications (CME, aphakia, glaucoma etc.) and in the management of drug therapy (topical treatment, classical immunosuppressives and biologicals) is elucidated. In this context, interesting LF topics (Do we treat cells, LF or both? Do high LF values necessarily demand for intensification of uveitis treatment? Does LF help us to decide when to stop our treatment?) are discussed.

**Joint Meeting: Improved monitoring and treatment of endogenous uveitis is modifying disease management/outcomes II**

• 4475

**Uveitis has become a precise clinical science: Multimodal investigational approach and management**

HERBORT C (1, 2)

(1) *Centre for Ophthalmic Specialized care b(COS), Lausanne*

(2) *University of Lausanne, Lausanne*

In recent years the appraisal of (posterior) uveitis has substantially improved. Classical investigational modalities such as fluorescein angiography (FA) and echography have been completed by an array of new precise and complementary investigational methods. Laser flare photometry (LFP) together with imaging methods including indocyanine green angiography (ICGA), optical coherence tomography (OCT), ultrasound biomicroscopy (UBM), fundus autofluorescence (FAF) together with functional tests such as microperimetry and computerized visual testing allow us to exactly determine the level of inflammation as well as morphological and functional involvement. This new comprehensive approach brought enhanced diagnostic possibilities as well as precise monitoring of disease evolution and impact of treatment.

• 4476

**Role of different imaging modalities in the differential diagnosis of inflammatory serous retinal detachments**

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Serous retinal detachment (SRD) may complicate a number of non infectious or infectious uveitic entities, including VKH disease, sympathetic ophthalmia, posterior scleritis, multifocal choroiditis, sarcoidosis, tuberculosis, and toxoplasmosis. Accurate detection and evaluation of inflammatory SRD relies on clinical examination and a multimodal imaging approach, including optical coherence tomography, fluorescein angiography, indocyanine green angiography, fundus autofluorescence, and ultrasonography, in selected cases. Moreover, multimodal imaging is useful in monitoring response to treatment. It also plays an important role in differentiating inflammatory SRD from central serous chorioretinopathy that may be induced or exacerbated by corticosteroid therapy.

• 4541

Welcome and introduction

*LANZETTA P  
Udine*

ABSTRACT NOT PROVIDED

• 4542

Aflibercept: innovation for wet AMD

*TADAYONIR  
Paris*

ABSTRACT NOT PROVIDED

• 4543

Optimising anti-VEGF therapy for wet AMD

*LANZETTA P  
Udine*

ABSTRACT NOT PROVIDED

**• 4621****Vitrectomized patients: Are they at risk for glaucoma?**

VANDEWALLE E  
Winksele

The prevalence of glaucoma has been suggested to be higher in vitrectomized patients than in the overall population. The mechanisms behind this reported higher prevalence are largely unknown but literature suggests that overall ocular physiology would be impaired in vitrectomized patients. The current theories on the importance of the vitreous body in ocular oxidative stress will be discussed. Furthermore, a review of the underlying diseases needing a vitrectomy and their relationship to glaucoma will be reviewed. Practical aspects such as decision making of combining cataract surgery to vitrectomy, membrane peeling and post-operative management of vitrectomized patients will be analyzed from the perspective of a glaucoma specialist.

**• 4622****Vitreous attachment to the optic disc: Strings pulling neurons?**

JONAS J  
Mannheim

The talk will present and discuss the epidemiology of a posterior vitreous detachment and its associations with clinical conditions

**• 4623****Retinal oxygen: the good, the bad and the unknown?**

STEFANSSON E  
Reykjavik

Ischemia is suspected in the pathophysiology of several eye diseases including glaucoma. The most direct way to study ischemia is through blood flow and circulatory studies and these have shown abnormalities in glaucoma and other eye diseases. However, blood flow changes per se do not necessarily indicate an unhealthy situation; blood flow indeed varies substantially in healthy tissues. The main metabolic consequence of ischemia is hypoxia and any harmful consequence of ischemia must include hypoxia at some point in time. Therefore, oxygen measurements are a direct way to study pathogenesis in ischemic diseases. Tissue damage would involve hypoxia and lack of hypoxia is not fully consistent with ischemic pathophysiology. The fact that hypoxia has to date not been found in glaucoma patients, suggests that ischemia may not be involved in the pathophysiology of glaucoma. The abnormalities in blood flow and oxygenation seen in glaucoma may be secondary to tissue atrophy, rather than the cause of the disease. Further studies, especially in patients with progressive glaucoma and high intraocular pressure will clarify the issue.

**• 4624****Intravitreal drugs: What is happening to the ganglion cells?**

PINTO L (1, 2)  
(1) Ophthalmology, Lisbon  
(2) Ophthalmology, Leuven

The number of patients undergoing intravitreal injections is growing exponentially. Long term treatments with anti-VEGF agents and/or steroids are now common in a number of retinal diseases. However, the significance of such treatment strategies on the pathogenesis of glaucoma or even how they may affect glaucoma management is not entirely clear. The possible impact of angiogenic and inflammation modulators in neuron cell survival and activity will be made. From a clinical point of view, it will be discussed if, how and which of these agents are more likely to affect intraocular pressure management.

## Joint Meeting: Keratoprostheses 1: Indications and surgical techniques

### • 4631

#### Boston type 1 KPros in India - Indications, contraindications and device modifications

SRINIVASAN B

Boston Keratoprosthesis is the most commonly used Keratoprosthesis worldwide. It is a collar button kind of Keratoprosthesis carried on a corneal graft. The prerequisites for a type 1 Boston Keratoprosthesis include the following

- 1) Bilateral corneal blindness
- 2) Reasonably healthy posterior segment
- 3) Well controlled Intraocular pressure or combine a shunt surgery with the Keratoprosthesis
- 4) Moist eye
- 5) Good blink
- 6) Well formed fornices
- 7) Committed to regular follow ups and Understands the potential risk involved in the surgery

The current indications for Boston type 1 Keratoprosthesis are

- 1) Multiple failed grafts
- 2) Limbal stem cell deficiency
- 3) Ocular burns( chemical/thermal)
- 4) Aniridia
- 5) Pediatric corneal opacities
- 6) Silicone oil induced keratopathy

Contraindications

- 1) Poor /Incomplete blink
- 2) Severely damaged lid apparatus post chemical injuries
- 3) Keratinized ocular surface (would be an indication for Boston type 2/MOOKP)

Design modifications like addition of more holes in the back plate, the use of the titanium locking ring, Use of a bandage contact lens and prophylaxis with antibiotics with the present design have improved the outcome and retention rates of the Keratoprosthesis. Further research in the form of a titanium back plate to reduce the rates of retroprosthetic membrane formation is being tried.

### • 4633

#### Preventing complications of OOKP surgery

AVADHANAM V, LIU C  
Brighton

Osteo-odontokeratoprosthesis (OOKP) is the procedure of choice for end stage ocular surface disease with severe dryness and keratinisation, and in those eyes with structural and functional lid abnormalities. The device retention rates and visual outcomes of the OOKP are far superior than any other keratoprosthesis. However, this device can have complications at every step during surgery and unto many years afterwards. Complications after the Stage 1 include: mucosal thinning and ulceration, mouth related, resorption of the implanted lamina and infections. Complications after the Stage 2 include: mucosal thinning and ulceration, mucosal overgrowth onto the optic, lid malpositions, lamina problems like resorption, tilting, infection, posterior segment complications like retinal detachment and vitreous haemorrhage. Glaucoma is a major cause of visual loss in a successful OOKP eye. Social and psychological complications can arise after visual restoration or the preexisting problems can continue. In addition, operative complications, systemic morbidity and anesthetic related problems do occur. A majority of the complications can be sight threatening if not rectified appropriately in a good time. They may be preventable or reducible with correct patient selection, improvements in preoperative and postoperative patient care and education. A sound surgical technique, reflective learning, harnessing the medical technology and multidisciplinary team approach can effectively reduce and treat the complications of OOKP surgery. Multidisciplinary team approach, having an experienced anesthetist and psychologist on board, and a continuous social support will prevent patient morbidity and optimise good outcomes.

### • 4632

#### Results, complications, and future developments of the Boston type 1 KPro

DE LA CRUZ J, CORTINA S  
Chicago, Illinois

The use of the Boston type 1 Kpro has increased significantly during the past decade not only in the US but around the World. Important device modifications together with changes in postoperative management have made this success possible. This presentation will review the current most relevant complications encountered in patients implanted with this device including endophthalmitis, retroprosthetic membranes, glaucoma and stromal melt. Strategies for their prevention and management will be discussed.

### • 4634

#### Biomimetic developments to improve the OOKP

AVADHANAM V, LIU C, LLOYD A, SANDERMAN S  
Brighton

While the alveo-dental tissue provides an excellent source for the Osteo-odontokeratoprosthesis (OOKP) skirt with highest biointegrability anteriorly and inertness posteriorly, it brings with itself a number of disadvantages like oral trauma, laminar resorption, complex surgical technique and multiple stages of the procedure. Additionally, edentulous patients and those with unsuitable teeth are left with the options of less successful allografts and tibial bone carriers for the KPro. Therefore, a synthetic skirt material which, could avoid the problems of tooth extraction, complications of allografts and immunosuppression will be advantageous. A lamina available off the shelf can drastically minimise the surgical procedure duration and stages, and has the potential for replacements. A number of materials have been proposed using Dacron skirts (Pintucci), ceramics, corals, bioactive glass and titanium. These materials have advantages and disadvantages. However, a synthetic material that mimics the alveo-dental tissue in porosity, mineral composition and biostability may prove suitable as a substrate for synthetic lamina.

• 4635

**Results of epicorneal keratoprostheses**

LIU C, AVADHANAM V

Brighton

Epicorneal keratoprostheses sit on the cornea with only the posterior optical part protruding through the cornea into the globe. Examples include the osteo-odontokeratoprosthesis (OOKP), tibial KPro, Pintucci KPro, and coralline KPro. Typically they have a biological cover usually buccal or labial mucosa. This paper examines the relative advantages and disadvantage of this family of devices, and their published results in terms of vision and retention.

• 4641

**Proteomic analysis of aqueous humor in retinoblastoma: Final results**

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 (1) Neurosciences and medical-surgical sciences, Siena  
 (2) Medical Biotechnologies, Siena  
 (3) Bioimaging institute and molecular physiology-CNR, Milan

**Purpose** Proteomics in ocular fluids is gaining rapidly relevance. Since 2009 we work on the analysis of proteic composition of the aqueous humor (AH) from retinoblastoma (RTB) patients and, in this work, we use the 2D-DIGE coupled to mass spectrometer to find new RTB markers.

**Methods** We enrolled 22 RTB patients (Reese-Ellsworth stage V or ABC classification group E) undergoing enucleation and 20 normal subjects undergoing cataract surgery (CTR). Five of 22 patients presented with associated secondary glaucoma whereas 17 had no secondary glaucoma; 8 of 17 patients with no secondary glaucoma received chemotherapeutic treatment with melphalan. Pools were analyzed using 2D-DIGE coupled to mass spectrometer. DIA and BVA were fully automated with 2D DeCyder® and statistical analysis was performed with EDA® module. Significant spots were In-Gel digested and identified with MALDI-TOF/TOF.

**Results** PCA analysis of the data underlines high distance between CTR pool and RTB pools. In particular the analysis of single protein shows that all the RTB pools are characterized by a high level of Crystallin and pro-inflammatory proteins and CTR pool are characterized by a high level of Epithelium Growth Factor.

**Conclusion** This study is the last step for the clarification of AH protein pattern in patients with RTB. Although we learn from literature that the presence of inflammatory processes is widely documented in late stages of pathology, however the presence of crystalline proteins could be either a new marker or an active part of the late stage tumor-induced panorama in RTB AH samples. Due to this, we think that several molecular biology studies are necessary to better understand involvement of this protein family in RTB pathological pathway.

• 4642

**Direct intraarterial (ophthalmic artery) chemotherapy for advanced intraocular retinoblastoma: Five years experience**

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 (1) Ophthalmology, Siena  
 (2) Neuroradiology, Siena

**Purpose** To report five years of conservative treatment for advanced retinoblastoma with the direct intra-arterial-ophthalmic artery infusion of Melphalan alone or Melphalan and Topotecan.

**Methods** 75 children (82 eyes) with advanced retinoblastoma (Stage D-E/VA -VB) were entered in phase two of one center open study-approved protocol of ophthalmic artery infusion (Italian intra-arterial protocol, approved by the Ethic Committee – University Hospital of Siena). Seven cases have been treated bilaterally. 40 eyes were first diagnosis (naïve) and 42 were relapses following chemotherapy and focal therapy and/or radiotherapy.

**Results** Cannulation of the ophthalmic artery was performed by a femoral artery approach using microcatheters (magic 1.5) while the children were under general anesthesia and anticoagulated. Chemotherapy (Melphalan alone or Melphalan and Topotecan) was infused into the artery over a 30-minute period (dose of 3-7 mg of Melphalan and 0.3-0.4 mg of Topotecan, according to the age and size of the globe). Local and systemic toxicity have been evaluated and documented.

**Conclusion** 75 children (82 eyes) with advanced retinoblastoma were eligible for the intra-arterial Italian Protocol. The 65.8% of all treated eyes is in complete remission. Superselective chemotherapy delivered through the ophthalmic artery can avoid enucleation, primary radiation or abuse of systemic chemotherapy.

• 4643

**Alternating intra-arterial and intravitreal chemotherapy for advanced intraocular retinoblastoma: First successful results without systemic chemotherapy**

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 (1) Ophthalmology/Ocular Oncology, Siena  
 (2) Neuroradiology, Siena  
 (3) Ophthalmology, Siena

**Purpose** Background: To describe the efficacy of intra-arterial chemotherapy combined with intravitreal chemotherapy for the treatment of advanced stage retinoblastoma

**Methods** The Authors report the medical records of four patients who presented with unilateral retinoblastoma, (Reese-Ellsworth stage Vb/group D of ABC Classification in the affected eye). They underwent clinical and ophthalmoscopic evaluation and MRI to exclude local and CNS dissemination. All 4 patients received 2 cycles (six infusions) of intrarterial chemotherapy (4-5 mg of melphalan/0.3-0.4 mg of topotecan), and from 7 up to 9 melphalan injections into the vitreous (10-30 µg in 0.05 mL).

**Results** Successful control of tumor masses and vitreous seeds was achieved in all cases at long-term (> 6 months) follow-up. Tumor control was 100% in all cases. Complications included: posterior lens opacities, acute ischemic papillitis, transient hypotonia, vitreous hemorrhage. There were no cases of orbital tumor recurrence or metastasis (follow-up range, 6 – 12 months).

**Conclusion** Sequential intra-arterial chemotherapy and intravitreal melphalan for advanced retinoblastoma allowed to provide retinal and vitreous seed control

• 4644

**The treatment of bilaterally advanced retinoblastoma: The Institut Curie experience**

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 (1) Institut Curie, Ophthalmology, Paris  
 (2) Institut Curie, Pediatric oncology, Paris  
 (3) Institut Curie, Pathology, Paris  
 (4) Institut Curie, Biostatistics, Paris

**Purpose** Since 2005 we have used more intensive chemotherapy, combined with intensive local treatment started at the third cycle in case of bilateral group D or group D+E bilateral retinoblastoma. We report the results

**Methods** All children identified with bilaterally advanced retinoblastoma were treated with 6 courses of 3 drugs. Local treatments including laser, cryotherapy and sometimes iodine plaque brachytherapy were started at the third cycle, synergistic with the chemotherapy. All tumors except the macular tumors were treated with laser during 5 to 20 minutes. The inferior periphery was treated with cryotherapy. After the end of the chemotherapy, close follow up was performed and additional local treatments were often necessary. Data concerning the initial findings, treatments and results were entered in the data base.

**Results**: the follow up ranges from 3 to 8 years with a mean follow up of five years. Between 2005 and 2010 23 group D eyes were treated in 16 children. 17 eyes are preserved without external beam (73%). One eye is lost to follow up. Visual acuity is available in 11 children ranging from counting fingers to 20/20 with a mean visual acuity of 20/50.

**Conclusion** Intravenous chemotherapy associated with intensive local treatments allows preservation of the eye with useful vision in a great percentage of group D eyes.

• 4645

**Molecular profiling of ocular surface squamous neoplasia identifies multiple DNA copy number alterations including recurring 8p11.22 amplicons**

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(1) *Ophthalmic Pathology, Riyadh*

(2) *Anatomic Pathology, Baltimore*

**Purpose** Conjunctival squamous cell carcinoma (cSCC) represent some of the most frequently encountered ocular surface neoplasms worldwide, and are particularly prevalent in Saudi Arabia. Our aim in this study is to uncover novel diagnostic biomarkers, as well as molecular pathways which can be targeted using new therapies.

**Methods** We analyzed DNA extracted from 14 snap frozen cSCC tumor specimens resected at KKESH using Agilent 180K high density oligonucleotide arrays, with 12 samples giving high quality hybridizations. We are now confirming increased gene dosage using the Nanostring platform and investigating mRNA expression at the 8p11.22 loci with quantitative PCR.

**Results** Of these 12, the number of clear regions of DNA loss ranged from 1 to 24 per tumor, while gains (including potential amplifications) ranged from 2 to 14 per tumor. Recurring aberrations were observed in chromosome 6, where the region 6p22.1-p21.32 was lost in 4 out of 12 specimens (33%), in chromosome 14, where the locus 14q13.2 was lost in 5 of the 12 samples (42%), and in chromosome 22, where 5 samples showed DNA loss and one DNA gain at 22q11.22. However the most frequent alteration was observed in chromosome 8, where the locus 8p11.22 was amplified in 9 tumors (75%) and lost in the other 3 samples (25%).

**Conclusion** We observed the most profound DNA alterations in the region of 8p11.22 which contains part or all of the ADAM1B, ADAM3A, and ADAM5p genes. It is also very near to the ADAM9 locus, which was recently found to be commonly amplified in oral squamous cell carcinoma (Plos One 2013;8:e54705).

• 4646

**IgG4-Positive orbitopathy**

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**Purpose** To give an overview of IgG4-positive orbitopathy. A newly described disease of the orbit.

**Methods** Critical review of the present literature demonstrated by a patient case.

**Results** The clinical appearance, high incidence of bilateral disease, association with lesions in other organs, and increased IgG 4 serum levels along with the histological findings of IgG4-positive cells, more background fibrosis, lymphoid hyperplasia, plasma cells and eosinophils are the characteristics of this disease.

**Conclusion** Patients with IgG4-positive cells in an orbital biopsy may have an orbital manifestation of IgG4-associated systemic disease

• 4647

**Pigmented basal cell carcinoma of the eyelid**

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**Purpose** While Basal cell carcinoma (BCC) is the most common malignant lesion of the eyelid, pigmented basal cell carcinoma of the eyelid are only rarely seen. Thus it is usually misdiagnosed clinically. The purpose of this presentation is to describe three cases with pigmented periocular BCC and to discuss its clinical and histopathological characteristics.

**Methods** A retrospective review was performed, of the files and the histopathologic slides of patients with pigmented BCC of the eyelid, who were treated in the Goldschleger Eye Institute, Sheba Medical Center, Tel Hashomer, Israel, during 2007-2012.

**Results** Fifty three patients were found with eyelid BCC diagnosed between 2007 and 2012, and three of them had pigmented BCC. Two of the tumors were on the lower lid and one in the medial canthus. All were uniformly or almost uniformly darkly pigmented. All were nodular and treated by complete surgical excision. The clinical diagnosis of two of the lesions was nevus.

**Conclusion** Pigmented BCC should be remembered among the differential diagnosis of pigmented lesions of the eyelid. It is interesting that our cases of pigmented BCC such as other cases described in the literature, were nodular or nodulo-ulcerative and not cystic or sclerosing types.

## • 4651

**Do we have any effective measure to minimize PCO?**

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In the past, posterior capsular opacification (PCO) was considered an inevitable, albeit relatively minor complication of cataract surgery. As technology has improved, the qualities of lens implants have changed. Multifocal intraocular lenses confer visual benefits but the optics are dependent upon a clear optical medium and accurate placement. The development of PCO can distort the centration and function of these advanced optical qualities, such as diffractive rings, rendering lenses suboptimal and in some cases intolerable. Therefore long-term quality outcomes are dependent on predictability and stability. The Bag-in-the-lens (BIL) implant is currently the most effective measure to prevent PCO. The technique depends on the construction of two comparable anterior and posterior capsulorhexes. The lens is suspended centrally, supported by the integrity of the capsule and seals away lens epithelial cells preventing postoperative opacification. This placement has also been proven to be highly resistant to decentration and rotation. As the uptake of multifocal lenses and patient expectations increase, we propose that the BIL approach is currently the most stable and dependable means of ensuring quality long-term multifocal outcomes.

## • 4652

**Is there any place for relaxing incisions in cataract surgery?**

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Brest

The advent of toric IOLs has certainly reduced the place of relaxing incisions. On the other hand, the use of femtosecond has significantly improved the easiness, the predictability and safety of the incisional procedure. Moreover the recent innovation of the "femtocataract" offers on some platforms the opportunity to combined relaxing incisions at the time of the crystalline lens removal. We will remind the different modalities for performing these incisions and will focus on disparities concerning the popularity of this surgery from one country to the other. The review of literature will provide the demonstration of the efficacy of incisions for correction of astigmatism especially in case of large cylindrical component such secondary astigmatism after triple procedure graft and cataract removal. We will also underline the interest of femtosecond laser in the refinement of the final outcome and will compare transepithelial versus intrastromal incisions. Of course the debate will be opened with toric IOLs and photoblation, that in some cases can be more complementary rather than competitive.

## • 4653

**What is important in present and future IOL materials & design?**

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IOL progress has followed 2 main lines: optical performance, and materials/design. This talk reviews their impact and possible future trends. IOL optics evolved from spheric monofocal to aspheric (+optionally toric) to multifocal; from refractive to diffractive multifocality, currently with several choices regarding add, bi- vs trifocality, etc. Additionally a number of designs attempt to reproduce the lens accommodative mechanism. However, none of the available options truly restores the physiological accommodation of the youth. The main drive for the evolution of IOL materials and designs has been implantability through an ever-decreasing incision width. While this may have created some compromises regarding materials and optical quality/stability, it seems unlikely that this process could revert. Next major issue is the prevention of PCO. Aspects as the posterior square edges and hydrophobic materials are widely assumed as useful to prevent PCO. However, a general proof is difficult due to the numerous factors involved (IOL & non-IOL) and the great variability of designs. IOL materials durability lags in a 3rd position of interest (apart from cases of IOL opacification) but is likely an emerging topic for the near future.

## • 4654

**Pentacam nuclear staging versus LOCS III grading for cataract classification**

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Cataract classification systems have been developed by several groups with different targets. Merriam and Focht for example aimed at classifying radiation damage, Eckerskorn and Hockwin included the aspect of multifactorial influences on cataract morphology, WHO wanted to develop a simple to use universal classification system for epidemiological studies and Chylack and coworkers tried to integrate morphology and colour. The Pentacam nuclear staging tries to correlate density to nuclear hardness which is yet another aspect. However, the stack of 50 Scheimpflug images provided by the Pentacam allows developing software which could objectively classify all cataract types by using 3D shape recognition. The development and application of such corresponding software will be discussed.

**• 4655****Is preoperative antibiotic use justified by evidence based medicine?***GRZYBOWSKI A (1, 2)**(1) Department of Ophthalmology, City Hospital, Poznan**(2) Department of Ophthalmology, University of Warmia and Mazury, Olsztyn*

Endophthalmitis is one of the most serious complications of the ocular surgery leading in most cases to a significant decrease in vision. The use of preoperative antibiotics and / or povidone-iodine has been advocated as an effective measure to prevent endophthalmitis, although it was never proved in a prospective trial. The additional benefit of topical preoperative antibiotics to povidone-iodine alone is controversial, and should be balanced with the risk of producing antibacterial resistance, the increasing problem of modern medicine. It was shown that 3 days preoperative use of major antibiotics is as effective as 1 hr preoperative use; povidone-iodine is as effective as antibiotic; single 5% povidone-iodine use is less effective than twice irrigation with the same solution; 10% povidone-iodine is more effective than 1% povidone-iodine. Moreover, preoperative antibiotic use did not lower the endophthalmitis rate. In conclusion, adding the antibiotic to povidone-iodine brings no clear benefit. The antimicrobial resistance is known to be produced by repeated, overused and extended use of antibiotics, and might lead to development of treatment resistant infections, including severe forms of endophthalmitis.

• 4661

**Prevention of autophagy activates inflammasome signaling in ARPE-19 cells treated with a proteasome inhibitor**

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**Purpose** Age-related macular degeneration (AMD) is the most prominent cause of severe vision loss in western countries. Inflammation is known to play a central role in the pathogenesis of AMD but the mechanisms are still largely unknown. Lately, the novel signaling pathway of intracellular multi-protein complexes called inflammasomes has been associated with the disease. In addition to inflammation, decline in the intracellular cleaning systems, i.e. autophagy and proteasomal degradation, is another hallmark of AMD. In the present study, we have studied the cross-talk between NLRP3 inflammasomes and autophagy.

**Methods** ARPE-19 cells were grown into confluence and exposed to the proteasome inhibitor MG-132. Thereafter, Bafilomycin A was added and the cell cultures were incubated for another 24 hours.

**Results** Our results show that the inhibition of lysosome acidification by Bafilomycin A increased the release of inflammasome-related cytokine IL-1 $\beta$  in MG-132-treated ARPE-19 cells. In addition, the amounts of inflammasome receptor and adaptor proteins, i.e. NLRP3 and ASC, respectively, as well as the activity of the executive Caspase-1 enzyme were increased.

**Conclusion** Our present results suggest that intracellular protein aggregates could induce the inflammasome activation in ARPE-19 cells especially in circumstances where autophagy is declined.

• 4662

**ELAVL1/HuR-mediated accumulation of SQSTM1/p62 during proteasomal inhibition in human ARPE-19 cells**

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**Purpose** Impaired protein degradation in retinal pigment epithelial (RPE) cells contributes to age-related macular degeneration (AMD) pathogenesis. SQSTM1/p62 protein is involved in autophagy and proteasome-mediated proteolysis, and expressed strongly under toxic stimuli. ELAVL1/HuR is a RNA-binding protein which, in response to various stressors, regulates gene expression, finally affecting key cellular functions. Main aims of our study were to investigate whether p62 mRNA would be a target of HuR protein, and whether a link between these two proteins exists in RPE cells. Finally, the effects of proteasome inhibition and autophagy induction on p62 and HuR levels in RPE cells were also evaluated.

**Methods** AlphaScreen technology and immunoprecipitation coupled with real-time qPCR were used to study the binding between HuR protein and p62 mRNA. ARPE-19 cells were treated with MG-132 (proteasome inhibitor; 5 $\mu$ M) and/or AICAR (AICA ribonucleotide, 5-aminoimidazole-4-carboxamide-1- $\beta$ -D-ribofuranoside; 2mM). P62 and HuR levels were analyzed by real-time qPCR and Western blotting. HuR silenced ARPE-19 cells and negative control cells were also employed.

**Results** MG-132 treatment up-regulates HuR, which binds p62 mRNA thus contributing, at post-transcriptional level, to the increase of p62 protein level in ARPE-19 cells. The addition of AICAR promoted cleansing by autophagy of p62, but not HuR.

**Conclusion** p62, whose mRNA represents a novel target of HuR, is positively regulated as a cellular response to proteasome inhibition. P62 is degraded by autophagy-mediated pathway, while HuR through proteasome. These findings may be relevant for AMD.

• 4663

**Pro-inflammatory cytokines induce apoptosis of human retinal capillary endothelial cells through downregulation of Hsp27**

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**Purpose** Retinal capillary cells undergo apoptosis in diabetes, but the mechanism is not clear. Pro-inflammatory cytokines are upregulated in the diabetic retina of humans and rodents. In this study, we have investigated the effect of pro-inflammatory cytokines on a small heat shock protein, Hsp27 in human retinal endothelial cells (HREC).

**Methods** HREC were cultured in the presence of pro-inflammatory cytokines, interferon- $\gamma$  (IFN- $\gamma$ , 50 and 100 units/ml), interleukin-1 $\beta$  (IL-1 $\beta$ , 10 and 20 ng/ml) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ , 10 and 20 ng/ml) for 48 hrs and in the presence or absence of high glucose (25 mM, HG). The roles of the kynurene pathway and NOS were determined by adding 20  $\mu$ M 1-methyl tryptophan (MT) and 500  $\mu$ M L-N $\omega$ -nitroarginine methyl ester hydrochloride (L-NAME), respectively to the culture medium.

**Results** HREC cultured in the presence of mixed cytokines showed a significant downregulation of Hsp27 at the protein and mRNA levels. This downregulation was not due to downregulation of the transcription factor, HSF-1. Mixed cytokines activated indoleamine 2,3-dioxygenase (IDO), enhanced kynurene production and increased the ROS content in HREC. MT inhibited the effects of mixed cytokines on Hsp27. Mixed cytokines upregulated NOS2 and increased levels of NO. A peroxynitrite donor and exogenous peroxynitrite drastically reduced Hsp27. The cytokine and HG-mediated downregulation of Hsp27 was accompanied by increased apoptosis of HREC.

**Conclusion** Our data suggest that pro-inflammatory cytokines induce ROS and NO formation, which through peroxynitrite production reduce Hsp27 and bring about apoptosis of HREC. These results suggest a novel mechanism for capillary cell death in diabetic retinopathy.

• 4664

**Characterization of rafts subtypes in monocytes of diabetic patients with and without neovascular complication**

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**Purpose** Diabetic retinopathy is a chronic inflammatory disease where the infiltration of plasma and neovascularization of the retina cause blindness. This disease affects millions of people in developed countries and early biological markers would better manage these patients.

**Methods** For this purpose we purified monocytes, key players of inflammation, blood of healthy and sick people. On the surface of monocytes, there are dynamic structures called rafts membrane microdomains, which there are two subtypes: flat rafts and caveolae rafts. They correspond to dynamic assemblies and ordered cholesterol and sphingolipids. We isolated these rafts membranes of monocytes and studied protein AT2 (angiotensin receptor II) and gp91 (major membrane subunit of NADPH oxidase, producing enzyme superoxide anions), potential markers of the severity of diabetic retinopathy.

**Results** Our results have shown a switch of cholesterol and sphingomyelin flat rafts to caveolae rafts. The analysis of the constituent proteins (flotillin and caveolin) and functional (AT2, gp91 and p47phox) have shown that these are mobile in the membrane in an inflammatory context. Subunits of NADPH oxidase seem to gather in caveolae rafts and more precisely when the enzyme is activated.

**Conclusion** The proteins of interest and the switch composition of lipid rafts therefore would appear to be an indicator of the progression of diabetic retinopathy and it would be interesting to provide a mechanistic explanation for the evolution of this chronic vascular disease to consider potential therapeutic targets.

• 4665

**Functional and molecular characterization of ex vivo cultured epiretinal membrane cells from proliferative diabetic retinopathy**

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**Purpose** To characterize the cell surface marker phenotype and function of ex vivo cultured cells growing out of human epiretinal membranes (ERMs) from proliferative diabetic retinopathy (PDR).

**Methods** All tissue collection complied with the Guidelines of the Helsinki Declaration. ERMs were obtained from vitrectomies due to intravitreal hemorrhage in PDR. Ex vivo cultivation under adherent conditions was performed in DMEM supplemented with FBS and the cell surface marker phenotype determined. Release of IL-6, IL-8 and TNFalpha was measured upon activation of the cells with TLR ligands and TNFalpha. The dynamics of the intracellular calcium was measured using fluorescent dye Fura-2 and imaged in response to mechano-stimulation.

**Results** The cultivated ERMs formed proliferating cell monolayers when cultivated ex vivo. These cells were negative for endothelial markers (CD31, VEGFR2), partially positive for hematopoietic (CD34, CD47) and mesenchymal markers (CD90, PDGFRb, CD73) and negative for CD105. IL-6, IL-8 and TNFalpha secretion could be measured upon activation of the cells by LPS, Poly I:C and TNFalpha. Mechano-stimulation of the outgrowing cells induced intracellular calcium propagation representing their functional viability.

**Conclusion** ERMs from PDR contain cells of hematopoietic and mesenchymal origin which have proliferative potential, release pro-inflammatory cytokines upon selective inflammatory stimulation and show functionality reflected through calcium dynamics upon mechano-stimulation.

• 4666 / T007

**The effect of hyperglycaemia on permeability and tight junction components in human retinal and choroidal endothelial cells**

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**Purpose** Diabetic retinopathy is the leading cause of preventable blindness in the working population. The main cause of visual loss in diabetic retinopathy is diabetic macular oedema caused by an increase in microvascular endothelial permeability. The aim of this project was to determine the effect of hyperglycaemia, *in vitro*, on human choroidal (hCEC) and retinal microvascular endothelial cells (hREC).

**Methods** Microvascular permeability was assessed through passage of dye through a confluent cell layer in the presence of hyperglycaemia. Microarray analysis and western blotting was used to compare the expression of selected tight junction molecules (Occludin, Claudin-5, JAM-A and JAM-C) and adheren junction (VE-Cadherin) molecules was compared between hCEC and hREC and with hyperglycaemia.

**Results** Hyperglycaemic conditions significantly increased the permeability in both hCEC and hREC. Microarray analysis and western blotting determined that the baseline hREC expression of occludin and claudin-5 was higher than hCEC. In hREC exposed to hyperglycaemia claudin-5, occludin and JAM-A were found to be reduced. None of the proteins were decreased by hyperglycaemia in hCEC.

**Conclusion** Although hyperglycaemia increased permeability in both hCEC and hREC, tight junction protein expression was only reduced in hREC, indicating a different mechanism of increased permeability in hCEC compared to hREC.

**Commercial interest**

• 4667 / T002

**Proteasome dysfunction in retinal pigment epithelium during aging contributes to the pathogenesis of Age-Macular related Degeneration**

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**Purpose** Age-related Macular Degeneration (AMD) is the leading cause of blindness in people older than 50 in developed countries, and is associated with formation of subretinal deposits (drusen) and damage to Bruch's membrane (BM) basal to the retinal pigment epithelium (RPE). It has been reported that activities of the ubiquitin-proteasome pathway (UPP) in retinal pigment epithelium decrease upon aging. Previous studies demonstrated that expression of ubiquitin in which lysine 6 is replaced by tryptophan (K6W-Ub) impairs the function of UPP. The main objective of this work was to investigate the hypothesis that chronically impairment of UPP in RPE contributes to some of the cardinal features of AMD, including drusens.

**Methods** To address this question ARPE-19 cells were infected with empty vector, K6W Ubiquitin mutant and Wild Type Ubiquitin. The drusen formation and accumulation in basal matrix was evaluated by confocal microscopy, using specific drusen markers such as APOE, while exosome realease was determined by western blot (such as CD63 and Tsg101) and flow cytometry following exosomes isolation by ultracentrifugation.

**Results** The results obtained showed that expression of K6W mutant Ub leads to an impairment of proteasome activity and an increased amount of exosomes release and drusen accumulation.

**Conclusion** In this study the data might provide a vaulable tool to elucidate the biological determinants of age-related proteolytic stress and its impacts on proteostasis in the retina.

• 4671

**The UK Sight Loss and Vision Priority Setting Partnership (SLV-PSP): Vision research questions prioritised by patients and health care professionals**

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**Purpose** The SLV-PSP process aims to identify research priorities that can be used to inform decisions of funding bodies and enhance the case for additional research funding.

**Methods** People were asked to submit questions about prevention, diagnosis and treatment of sight loss and eye conditions that are most important to them. The survey was available online, by phone, post and alternative formats (Braille and audio). The partner organisations were Fight for Sight, the College of Optometrists, NIHR Biomedical Research Centre for Ophthalmology, The Royal College of Ophthalmologists and Vision 2020. Over 30 charitable and professional organisations promoted the survey.

**Results** 2,200 people responded generating 4,461 questions. 65% of respondents had sight loss or an eye condition. 17% were healthcare professionals (ophthalmologists, optometrists, orthoptists, ophthalmic nurses, opticians, social care/ rehabilitation). The questions were allocated to 12 eye disease categories before subsequent prioritisation of the top 10 at 12 workshops (questions at <http://fightforsight.org.uk/sightlosspsp>). The categories were ARMD, cataract, corneal and external, glaucoma, inherited retinal, neuro-ophthalmology, ocular cancer, ocular inflammatory, refractive error and ocular motility and vitreoretinal/ocular trauma.

**Conclusion** Analysis of demographic data for respondents indicates that the sample is generally representative of the UK population. The number and diversity of conditions represented in responses is distributed across the major conditions and fits projected population incidence.

• 4673

**The KORA-AGE eye study: Eye diseases in the elderly**

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**Purpose** To estimate the prevalence of major age-related eye diseases in a population-based regional study in Southern Germany.

**Methods** 822 randomly selected persons (age 68-96 years) from the KORA AGE study were asked in 2012 in a standardized interview for the presence of major eye disorders like cataracts, glaucoma and age-related macula degeneration (AMD). In case of any positive reply, the ophthalmologist in charge was asked for validation and specification of any eye disease.

**Results** 465 persons reported any eye disorder (57%); 71% of them could be validated and specified. There were 182 confirmed cases of cataracts, 7 of glaucoma and 5 of AMD. Additionally, there were 52 cases of cataracts and AMD, also 54 cases of cataract and glaucoma and 11 cases of cataract, glaucoma and AMD. In 62% cases cataracts developed prior to any of the other eye diseases. Adjusted for age, women had a significantly higher risk for cataracts (OR = 1.72) and for AMD (OR = 1.94) than men; no gender-specific difference was observed for glaucoma. Among patients with cataracts, 69% had lens surgery.

**Conclusion** We confirmed cataracts as the major age-related eye diseases; however, the number of glaucoma and AMD were surprisingly low. Further analyses are planned to identify risk factors and to show how eye diseases are independent risk factors for increased frailty and disability in the aged. This study was supported by the German Federal Ministry of Education and Research (BMBF) within the programme "Healthy Ageing" (FKZ 01ET1003)

• 4672

**Visual acuity thresholds associated with activity limitations in the elderly. The POLA study**

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**Purpose** Activity limitations, which induce loss of autonomy in the elderly, are a major public health problem. We investigated the associations between objectively determined visual impairments and activity limitations, and assessed the visual acuity thresholds associated with these restrictions.

**Methods** The study sample consisted of 1887 people aged 63 years and over from a population-based cohort. Visual impairment was defined according to the WHO definition. Multivariate logistic regressions were used to estimate the associations between vision and Instrumental Activities of Daily Living (IADL) limitations. Using receiver-operating characteristic (ROC) curves, we identified visual acuity thresholds that maximized the Youden index ( $[Sensitivity+Specificity]-1$ ) for predicting IADL limitations.

**Results** After adjustment for potential confounders, moderate to severe visual impairment and mild visual impairment were strongly associated with IADL limitations (odds ratio [OR]=3.49; 95% confidence interval [CI]=1.93, 6.32 and OR=1.77; 95% CI=1.07, 2.91, respectively). Visual acuity was a strong predictor of IADL limitations, with an area under the ROC curve of 0.72 (95% CI=0.68, 0.76). The best discrimination between subjects with or without IADL limitations (global, physical, cognitive) was obtained for visual acuities around 20/40-20/50.

**Conclusion** This study confirms major increased risk for IADL limitations in subjects with moderate to severe visual impairment. In addition, it suggests that milder visual impairments (in particular below 20/40) may also be related to an increased risk for IADL limitations and should be considered for early medical intervention, before the decline of the subjects' autonomy.

• 4674

**The effects of interleukin-8, VEGF and CFH polymorphisms on the long-term response to bevacizumab therapy in exudative age-related macular degeneration**

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**Purpose** To study the effects of IL-8, VEGF, CFH, complement component C3 and LOC387715 single nucleotide polymorphisms and neovascular lesion characteristics on the response to intravitreal bevacizumab treatment in exudative age-related macular degeneration (AMD).

**Methods** Fifty patients with treatment naïve exudative AMD were recruited to this two-year prospective follow-up study, and treated with bevacizumab on their once-a-month visits when sub- or intraretinal fluid in OCTs, or a new hemorrhage was detected. Visual acuities (VA) and contrast sensitivities (CS) were assessed on every visit, and fluorescein (FA) and indocyanine green (ICGA) angiographies recorded at baseline, and after one and two years of follow-up. Blood samples were collected for genotyping.

**Results** VEGF -2578A/C and CFH Y402H polymorphisms and baseline lesion size in ICGA associated with the number of needed reinjections during the follow-up, and IL-8 -251A/T associated with the disappearance of fluid in OCTs. The alleles A in IL-8 -251A/T, A in VEGF -2578A/C, and C in CFH Y402H had a cumulative effect on the presence of fluid in OCTs. A marked difference existed between patients having 0-2 risk alleles compared to those having 3-6 risk alleles ( $P=0.00002$ ). An association was also detected between the VEGF -2578A/C polymorphism and CS gain ( $P=0.014$ ). During the first year VA change correlated negatively with the number of visits when cystic changes were detected in the OCTs ( $r=-0.366$ ,  $P=0.009$ ), but the association did not persist during the second year.

**Conclusion** VEGF -2578A/C, IL-8 -251A/T, and CFH Y402H seem to affect the persistence of fluid in the macular area during bevacizumab therapy of exudative AMD.

## • 4675

**Functional characterization of retinitis pigmentosa causing RPGR mutations**

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**Purpose** Retinitis pigmentosa (RP) is a progressive outer retinal dystrophy, affecting 1/3500 individuals in most populations. X-linked RP (XLRP) is one of the most severe forms of human retinal degeneration, as determined by age-of-onset and progression. Mutations in the retinitis pigmentosa GTPase regulator (RPGR) gene, responsible for the major subtype of XLRP, are the most common single cause of RP, accounting for 6-20% of all cases. We aim to investigate the functional role of RPGR mutations in the pathogenesis of XLRP.

**Methods** Different RP-causing RPGR mutations were cloned into pCEP-4 expression vector and transfected into retinal pigment epithelium (RPE) 1 and IMCD3 cell lines. RPGR expression was detected by immunocytochemistry and western blotting. The pathological effects caused by RPGR mutations were characterized by immunocytochemistry.

**Results** The average length of cilia in RPE1 cells with over-expression of mutant RPGR are around 50-60% shorter compared to that in cells with over-expression of wild type RPGR. Cells with over-expression of RPGR mutant proteins exhibited stronger actin filaments. The frameshift RPGR mutations also cause aggregations in IMCD3 cell lines indicating that the mutant proteins are misfolded which may cause toxicity.

**Conclusion** Mutant RPGR proteins have functional role in cilia defect through regulating actin remodelling.

## • 4676 / T073

**Influence of refractive error and axial length on retinal vessel geometric characteristics**

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**Purpose** To evaluate the influence of refractive error and axial length (AL) on retinal vascular network geometry measurements in an adult Asian population.

**Methods** This was a population-based, cross-sectional study on 2882 persons with diabetes in the Singapore Malay Eye Study (SiMES). Spherical equivalent refraction (SE) was assessed using an autokeratometer and subjective refraction. AL was measured by IOL Master. Retinal vascular caliber, tortuosity, and branching characteristics were quantified from retinal fundus photographs using a semi-automated computer-assisted program according to a standardized protocol.

**Results** In multivariate analyses adjusting for age, gender, education, smoking, blood pressure, diabetes status, and anti-hypertensive medication use, longer AL and more myopic refraction were associated with narrower retinal arterioles and venules.( $p \leq 0.001$  for all) and less tortuous (straighter) arterioles.( $p < 0.001$  for both). Longer AL and more myopic refraction were also associated with increased branching coefficients in both arterioles ( $p < 0.001$  for both) and venules.( $p = 0.02$  and  $p < 0.001$  respectively). Longer AL and more myopic refraction were associated with more acute branching angles in arterioles ( $p < 0.001$  for both) but not venules.

**Conclusion** Myopic refractive errors and longer AL are associated with narrower retinal arterioles and venules, less tortuous arterioles, and increased branching coefficients in both arterioles and venules. These findings provide insights into ocular blood flow in myopia, and also suggest that future studies evaluating these retinal parameters should account for the influence of AL and refractive error.

## • 4677 / T074

**Early dietary therapy in preventing progression of retinopathy in long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency caused by the homozygous G1528C mutation**

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**Purpose** To evaluate the efficacy of the early start of a low fat, high carbohydrate diet in preventing progression of pigmentary retinopathy, a characteristic feature of long-chain 3-hydroxy-acyl-CoA dehydrogenase (LCHAD) deficiency, a mitochondrial long-chain fatty acid  $\beta$ -oxidation defect.

**Methods** 13 Finnish patients with early diagnosis of LCHAD deficiency caused by the homozygous G1528C/Q510Q common mutation, were followed up to 20 years (median 6 y). An ophthalmic examination was performed every 1-2 years including BCVA, colour vision, axial length, fundus colour photography, OCT, and ERG.

**Results** 11 out of 13 patients had mild pigmentary changes already in their first ocular exam (median age of 6 mos), while 2 had pale newborn retina. The initial fundus changes were most often pigment granularity in the posterior pole, hyperpigmented macula and pale fundus. In 12 patients, with good compliance of the dietary therapy, the pigmentary changes progressed only mildly or not at all, remaining at stage II of retinopathy with no atrophy and visual compromise. The only patient with an overall poor outcome, had progression of the chorioretinopathy to stage III, presenting atrophy in the posterior pole with relative sparing of the macula and far periphery.

**Conclusion** Our long-term ocular follow-up data are promising and suggest that the low-fat, high-carbohydrate diet started during the first months of life may delay or even halt the progression of the pigmentary retinopathy and possibly prevents the visual handicap in LCHAD deficiency.

## • 4711

**Macular choroidal thickness profile in healthy population measured by swept-source optical coherence tomography**

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**Purpose** To determine choroidal thickness (CT) profile in healthy population by swept-source longer-wavelength optical coherence tomography (SS-OCT).

**Methods** Cross-sectional, non-interventional study. The macular area of 276 eyes from 154 healthy patients was studied with an SS-OCT prototype system. A horizontal CT profile of the macula was created by manually measuring subfoveal CT (SFCT, from the posterior edge of retinal pigment epithelium to the choroid/sclera junction). Three further determinations were performed every 1000 µm nasal to the fovea and five more every 1000 µm temporal to the fovea. CT was independently determined by two observers.

**Results** SS-OCT allowed visualization of CT in all cases (100%). Mean SFCT was  $301.89 \pm 80.53$  µm (95% confidence interval: 292.34 to 311.43). Mean horizontal macular CT (MCT) was  $258.69 \pm 64.59$  µm (95% confidence interval: 251.04 to 266.35). Mean SFCT was  $325.6 \pm 51.1$  µm in 0 to 10 years,  $316.7 \pm 90.1$  µm in 11 to 20 years,  $313.9 \pm 80.3$  µm in 21 to 40 years,  $264.6 \pm 79.3$  µm in 41 to 60 years and  $276.3 \pm 88.8$  µm in more than 60 years ( $P < 0.001$ ; Anova test). Mean horizontal MCT was  $286.0 \pm 43.5$  µm,  $277.7 \pm 68.2$  µm,  $264.0 \pm 61.9$  µm,  $223.4 \pm 62.2$  µm and  $229.7 \pm 66.1$  µm for 0 to 10, 11 to 20, 21 to 40, 41 to 60 and >60 years respectively ( $P < 0.001$ ; Anova test). The CT profile was different for each age group.

**Conclusion** Macular CT profile in healthy population is similar between different age groups with thicker CT in young people.

## • 4712

**Combination of ranibizumab and navigated retinal photocoagulation in diabetic macular edema, compared to ranibizumab mono-therapy: Twelve month results**

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**Purpose** To evaluate the number of anti-VEGF injections needed in DME patients using a protocol with navigated laser

**Methods** A consecutive series of 76 eyes with DME were included and randomized into 3 groups: 1. Ranibizumab monotherapy ( $n=27$ ; using an observation and retreatment paradigm for anti-VEGF therapy that is compliant with the European Public Assessment Report (EPAR, European Medicines Agency) for ranibizumab), 2. Three consecutive monthly ranibizumab injections followed by navigated laser therapy, then application of the observation and retreatment paradigm as in group "1" ( $n=15$ ), 3. Monthly ranibizumab injection until central retinal thickness (CRT) was reduced to  $450\mu\text{m}$  (Spectralis OCT) then navigated laser therapy followed by the application of the observation and retreatment paradigm as in group "1" ( $n=34$ ). Subjects were followed monthly (best corrected Visual acuity (BCVA), CRT) for 12month, to assess the number of anti-VEGF injections required to maintain stable clinical improvement.

**Results** After 12 month BCVA increased and in all three investigated groups significantly (group 1:  $6.3 \pm 6.77$ ; group 2:  $2.7.1 \pm 8.22$ ; group 3:  $7.4 \pm 7.53$  letters). To achieve these results, after an upload of three consecutive monthly applied ranibizumab injections, ranibizumab mono-therapy group (group 1) needed  $5.2 \pm 3.2$  injections. In contrast, group 2 and 3 needed significantly less ranibizumab injections ( $0.5 \pm 0.8$  and  $0.8 \pm 1.1$ ,  $p < 0.001$ ).

**Conclusion** Combining anti-VEGF with navigated laser showed similar visual improvements compared to anti-VEGF monotherapy with significantly fewer injections.

*Commercial interest*

## • 4713

**High doses fatty acids for improving vision in patients with dry and wet macular degeneration**

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**Purpose** To evaluate the effect of high doses EPA/DHA for dry and wet macular degeneration

**Methods** 40 eyes with dry macular degeneration (6 months follow up) and 43 eyes with wet macular degeneration (1 year follow up) were treated with 5g/day of EPA/DHA. Results of the visual acuity and/or mfERG and/or OCT scans were noted.

**Results** Visual acuity for dry macular degeneration patients improved by one or more lines in 80% of eyes at 6/52, 88% at 3/12 and 100% of eyes at 4.5/12. The mean macular oedema for wet macular degeneration was reduced from 487 to 280µm at 1 year and the mean visual acuity gain was 1.4 lines at 1 year. The average number of anti-VEGF injections for wet macular degeneration injected was 0.77 over 1 year.

**Conclusion** Innovative therapy of using high doses fatty acids to improve vision in patients with dry and wet macular degeneration.

## • 4714 / F038

**Intravitreal afibercept (IVT-AFL) for macular edema secondary to CRVO: Results of COPERNICUS and GALILEO studies**

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**Purpose** Evaluate efficacy and safety of IVT-AFL for treatment of macular edema due to CRVO.

**Methods** Patients randomized to IVT-AFL 2-mg (IVT-AFL 2q4) or sham monthly for 24 weeks. COPERNICUS: all patients received IVT-AFL PRN from week 24-100 (IVT-AFL 2q4 PRN and sham IVT-AFL PRN). GALILEO: IVT-AFL PRN was administered to IVT-AFL group from week 24-76 and sham group from week 52-76.

**Results** Proportion of IVT-AFL 2q4 PRN and sham IVT-AFL PRN patients gaining  $\geq 15$  letters was 56.1% vs 12.3% and 49.1% vs 23.3%, respectively, at week 24 and 100 (COPERNICUS) and 60.2% vs 22.1% and 57.3% vs 29.4%, respectively, at week 24 and 76 (GALILEO; all  $P < 0.001$ ). Mean change in BCVA was +17.3 vs -4.0 ( $P < 0.0001$ ) and +13.0 vs +1.5 ETDRS letters ( $P = 0.007$ ) at week 24 and 100 (COPERNICUS) and +18.0 vs +3.3 ( $P < 0.001$ ) and +13.7 vs +6.2 letters ( $P = 0.007$ ) at week 24 and 76 (GALILEO) in IVT-AFL 2q4 PRN and sham IVT-AFL PRN groups, respectively. Mean reduction from baseline CRT was 457.2 vs 144.8 µm ( $P < 0.001$ ) and 390.0 vs 343.3 µm at week 24 and 100 in COPERNICUS and 448.6 vs 169.3 µm ( $P < 0.0001$ ) and 389.4 vs 306.4 µm at week 24 and 76 in GALILEO in IVT-AFL 2q4 PRN and sham IVT-AFL PRN groups, respectively. Most frequent ocular SAEs in IVT-AFL 2q4 PRN group were cataract (COPERNICUS) and macular edema (GALILEO). APTC ATEs occurred only in COPERNICUS (2 (1.8%) IVT-AFL, 2 (2.7%) sham).

**Conclusion** IVT-AFL treatment resulted in early, sustained VA gains. Maintenance of VA gains with an extended treatment interval after initial monthly dosing depended on monthly monitoring. Long-term fixed dosing regimens may be a better treatment paradigm.

*Commercial interest*

**• 4715****Direct versus indirect ophthalmoscopy: Medical student assessment of retinal pathology**

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**Purpose** Fundoscopy by non Ophthalmic specialists is typically performed by direct ophthalmoscopy. This challenging skill gives a magnified, monocular image. In contrast, indirect ophthalmoscopy as regularly used by Ophthalmologists allows a wide field, binocular view. The aim of the study was to test the hypothesis that compared to direct ophthalmoscopy, indirect ophthalmoscopy is an easier technique to learn and that the correct diagnosis of fundus pathology is more readily achieved.

**Methods** The design was a prospective cohort study, conducted on 50 third year medical students, at a large inner city hospital. Participants attended a practical teaching session on both techniques. Students were then randomly allocated to either ophthalmoscopy technique and scored on their ability to recognise common fundus pathology in a group of patients. Student perception of the technique was also ascertained.

**Results** The number of surveys filled out for first and second session was 98% and 86% respectively. After the initial teaching session, the majority of students reported being 'moderately' confident in using both techniques (direct 52%; indirect 35%), but overall 59% preferred direct ophthalmoscopy. When asked to identify common retinal pathology, it was found that students allocated to direct ophthalmoscopy were more likely to correctly identify the pathology although not statistically significant ( $U$  value 9.5,  $p \leq 0.05$ ).

**Conclusion** Although indirect ophthalmoscopy provides better views of the retina, students are more confident using direct ophthalmoscopy. Furthermore, there is no significant difference in ability to diagnosis retinal pathology between either techniques.

**• 4721****Modern Trabeculectomy: Technique, and results from a UK multicentre study**

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Efficacy and safety of current trabeculectomy surgeryMethods: 428 patients with OAG and no previous glaucoma surgery from 9 units were evaluated. All cases had 2 yr minimum follow up.Main outcomes: IOP, VA, complications and interventions. Reoperation for glaucoma (except needling and resuturing) and loss of light perception were classified as failures.Results: Antifibrotics were used in 400 (93%) cases; MMC in 271 (63%), 129 had 5-FU (30%). At 2 years, IOP (mean  $\pm$  SD) was  $12.3 \pm 4.3$  mmHg, 370 (86%) had IOP  $\leq 21$  mmHg without medication; 405 (95%) achieved an IOP  $\leq 21$  mmHg overall. IOP  $\leq 18$  mm Hg was achieved by 352 (82%) without treatment and 373 (87%) overall. By TGT criteria: 310/365 (85%) had complete success at 2 yr and 335/365 (92%) achieved qualified success. Postoperative treatments: suture manipulation 43%, resuturing 5.1%, bleb needling 16%, postop 5FU 27% and cataract extraction in 31%. Visual loss of more than two lines occurred in 24 (5.6%). 31 (7.2%) had late hypotony (IOP  $< 6$  mmHg). 2 patients (0.5%) developed blebitis. One patient had endophthalmitis at 3 wks (0.2%). We show good trabeculectomy outcomes with low rates of complications can be achieved but intensive postoperative care is required.

**• 4722****Viscocanalostomy and canaloplasty : Technique and results**

SUNARIC MEGEVAND G  
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Non penetrating surgical procedures involving Schlemm canal represent a new challenge in glaucoma surgery. While allowing good IOP control this technique avoids major complications known with Trabeculectomy and avoids the need for a subconjunctival filtering bleb which shunts aqueous to nonphysiological routes. Viscocanalostomy is characterized by the enlargement of Schlemm's canal with viscoelastic near the surgical margins, in order to enhance aqueous egress through the ostia of the canal and then out of the eye via aqueous collector channels. Since the first description of Viscocanalostomy by Stegman several improvements have been made in the technique of the procedure resulting finally in the 360° catheterization of the Schlemm's canal, a procedure called Canaloplasty. The technique, personal and published results, advantages and disadvantages of these procedures will be discussed.

**• 4723****Novel glaucoma surgery techniques**

ZEYEN T  
*Leuven*

Novel glaucoma surgery techniques can be divided into ab-interno gonio-surgeries and ab-externo procedures involving the conjunctiva. An overview of the new procedures will be provided with emphasis on their efficacy and complications. The evidence of the available studies will be presented and an attempt will be made to clarify the role of the novel procedures within our glaucoma treatment algorithm.

**• 4724****UK National Ophthalmology database: optimising surgical safety**

JOHNSTON R  
*Gloucestershire*

The UK National Ophthalmology Database was established under the auspices of The Royal College of Ophthalmologists to collate anonymised patient data from electronic medical record systems that collect nationally agreed datasets for the purposes of national audit, research and revalidation of doctors. Following appropriate permissions data conforming to nationally agreed datasets were remotely extracted and anonymised. In 2011 data were extracted from 31 centres on a total of 382,275 patients including trabeculectomy operations. National audits and inter-surgeon and inter-institutional comparisons can be automated and allow surgeons to view their performance in the context of their anonymised peers.

## Joint Meeting: Keratoprostheses 2 - Tissue and bioengineering approaches

### • 4731

#### **Microbiological, Chemical, and Mathematical Analysis of Alexidine-Polyethylene Interaction: Implications for the Fusarium Keratitis Epidemic of 2004-2006**

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Our previous studies indicated that alexidine permeates into the walls of heated ReNu plastic bottles, thereby diminishing its concentration within the solution, thus allowing *Fusarium* growth. Using liquid chromatography-mass spectroscopy (LCMS), alexidine levels were measured in heated/unheated ReNu bottles stored for various time periods. These data were plotted as decreasing alexidine concentrations vs. time; ratios of the areas under the curves were calculated and compared with data derived from Fourier transform infrared (FTIR) spectroscopy absorptions of methanol extract evaporate residues of formerly alexidine-exposed bottle walls. FTIR studies showed that there was approximately 3.1 times more alexidine in the wall of the heated than the room temperature-stored bottle. The LCMS study showed that there was approximately 3.2 times more alexidine in the walls of heated bottles. Alexidine levels correlated closely with timed and dilution microbiological studies. Decreasing alexidine levels with time and heat strongly suggest alexidine-polyethylene interaction as the pharmaceutical failure mechanism of the worldwide *Fusarium* keratitis epidemic of 2004-2006.

### • 4732

#### **Biomaterials in ophthalmic applications - IOL's and keratoprostheses**

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- (2) Halle
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Biomaterials can be regarded as synthetic or non-living native materials used in therapeutic approaches. This direct contact with body tissue manifests itself in a wide spectrum of chemical, physical and biochemical interactions. In ophthalmology, biomaterials find their application in the development of intraocular lenses (IOL's) and keratoprostheses. The chemical nature of these materials encompasses the full spectrum of physico-chemical properties of matter; e.g. hydrophilic and hydrophobic poly(meth) acrylates, silicones and fluorinated polymers. Concordantly, the intended use and functionality of the implant necessitates the strict selection of the desired chemical and physical characteristics of the materials to be used, if not the de-novo creation of suitable substances. In addition, modification of surfaces or the creation of gradual changes in material properties by co-polymerizations or alternative means of tailoring peculiar aspects of the device may be required to achieve reasonable or functional substitution of the diseased tissue, all the while maintaining ease of the device's usage by the caring physician and, above all, uncompromised patient's safety in the intended use of the device throughout its lifecycle.

### • 4733

#### **In vivo integrity of intra-corneal bioengineered discs in rabbit models**

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**Background:** We have previously reported the successful integration and safety of bioengineered materials as corneal substitutes in human models. Despite the promising results as corneal implants, more elastic and robust materials are required for use as thin intra-corneal lenses to withstand surgical manipulation for corrective surgery and improved vision. Most of the existing corneal inlays are made of synthetic materials. Here we describe the potential of bioengineered materials for vision correction. **Objectives:** to develop bioengineered materials as inlays within the corneal tissue as well as evaluating the in vivo integrity and integration of the materials in rabbit models. **Methods:** Bioengineered inlays were prepared from collagen and tested for their physical and biological properties. A femtosecond laser was used to cut 100 micron thick discs of mid-stromal tissue from corneas of 20 rabbits and replaced with bioengineered inlays. **Results:** The new materials demonstrated improved mechanical properties while maintaining their clarity and biocompatibility. The bioengineered inlays retained their shapes, thickness, and clarity 8 weeks post-surgery in rabbits.

**Commercial interest**

### • 4734

#### **Nanofibrous scaffolds for corneal tissue engineering**

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This contribution will report on nanofibrous scaffolds for corneal tissue engineering. By electrospinning, biodegradable nanofibers can be created that form a semitransparent layer. This can be used as a substrate for further cell seeding procedures. It is the goal to use this scaffold in ocular surface reconstruction.

• 4735

### Ocular surface reconstruction

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The ocular surface is a highly specialised environment with complex homeostatic mechanisms. Severe ocular surface disease can cause significant morbidity through recurrent epithelial breakdown, predisposition to infection and blindness. Ocular surface reconstruction (OSR) strategies aim to restore sufficient ocular surface function to permit the maintenance of an intact transparent epithelium. OSR begins by addressing the quality and volume of tears. Next the position and closure of the eyelids are evaluated and addressed. Only once these factors have been addressed can attention be turned to surgical reconstruction of the ocular surface. The algorithms that guide the choice and timing of surgical interventions are becoming more clear. These universally advocate transplantation of limbal epithelial stem cells prior to surgical replacement of stroma with or without endothelium. Results for autologous ex-vivo expanded limbal stem cell transplants in chemical injuries are excellent. Results for allogeneic grafts are no better than older whole limbal transplants such as keratolimbal allografts. Ex-vivo cultured autologous oral mucosal stem cells offer an alternative in bilateral disease but such grafts are less optically transparent.

• 4736

### Immunomodulatory effects of gene therapy in composite corneal grafts

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Corneal allografts overexpressing anti-apoptotic protein p35 in BALB/c mice have shown significantly reduced delayed-type hypersensitivity. However, the precise mechanisms responsible for altered immune response have been unclear. Based on previous data, we hypothesized that expression of p35 in corneal epithelium reduces the priming of T lymphocytes in draining lymph nodes after transplantation. In our results, mixed lymphocyte reaction with C57BL/6 splenocytes revealed statistically decreased frequency of CD4+ T cells in allograft group treated with p35 compared to other allogeneic groups. These findings provide new immunological insights on priming of T cells modified by genetically treated composite allograft to improve transplant survival.

## Course 18: Femtosecond laser cataract surgery

### • 4751

#### The basics of femtosecond laser cataract surgery

NAGYZ

*Semmelweis University, Department of Ophthalmology, Budapest*

Femtosecond lasers have been used for corneal flaps for years. Recently the problems of beam delivery through the ocular optic media has been solved. Precise incisions can be performed within the crystalline lens (fragmentation or liquefaction), on the anterior capsule and possible on the posterior capsule as well (capsulotomy) and in the cornea (customised geometry, size and position of the wounds). During the lecture the basics of femtolaser cataract surgery will be shown.

### • 4752

#### Current available platforms for femtosecond laser cataract surgery

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Different femtosecond laser platforms are nowadays available. Main differences as the particular approaches in anterior segment imaging techniques to focus the laser pulses precisely, interface designs to efficiently and safely dock the patient's eye and software options in each platform will be presented and discussed.

### • 4753

#### Femtosecond cataract surgery: The basic procedure and surgical details

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Femtosecond lasers (FsL) have already proven to be a useful tool to improve efficacy and safety in cataract surgery. This section of the course will review the basic steps of FsL-assisted cataract surgery, the learning curve and transition, as well as the different variants and maneuvers of the procedure as compared to traditional phaco.

### • 4754

#### Proven advantages of femtosecond laser cataract surgery

NAGYZ

*Semmelweis University, Department of Ophthalmology, Budapest*

Results with femtosecond laser cataract surgery especially with premium lenses (multifocal and accommodative lenses) will be presented. In peer-reviewed articles was shown the superior results with better posterior chamber lens position, less PCO formation, more favorable results with postoperative higher order aberrations and vision quality will be discussed. The method also useful after ocular trauma, in eyes with loose zonules, in PEX syndrome, in white tumescent cataract and possibly in pediatric cataract. These result will be presented during the course.

**Course 18: Femtosecond laser cataract surgery**

• 4755

**Complications & special cases of femtosecond laser cataract surgery**

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This section of the course will review the specific aspects of FsL-assisted cataract surgery, including the evidences on the increased efficacy, precision and safety, the possible complications, and a number of special cases and situations as described by video presentations.

• 4761

**Endothelial graft precutting from the epithelial and endothelial side with the femtosecond laser, on cornea stored in a new corneal bioreactor**

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**Purpose** To compare femtosecond laser endothelial graft precut from epithelial and endothelial side, on corneas stored in a new corneal bioreactor

**Methods** Human corneas unsuitable for transplantation for serological reasons and procured by the Auvergne-Loire eye bank (Saint Etienne, France) were used after informed consent of the relatives, as authorized by French bioethics laws. Femtosecond laser lamellar cuts were realized on corneas stored in a new corneal bioreactor, patented by our laboratory, designed to maintain a constant endothelial pressure and to permit an epithelial applanation without opening the device. Lamellar cuts of several thickness were realized with corneal epithelium or corneal endothelium side up, but always with epithelial applanation. OCT images (Casis SS1000, Tome) were realized in the bioreactor before and after each cut. Endothelial viability was determined by using a triple Hoechst/Ethidium/Calcein labeling (HEC, IOVS Pipparelli 2011) coupled with image analysis of the whole endothelial surface immediately after the cut. Surface roughness was assessed by scanning electron microscopy

**Results** This study demonstrates the possibility to realise femtoseconde laser endothelial graft precut directly in a dedicated storage device. First results of endothelial viability in function of lamellar graft thickness and lamellar cut side will be presented as well as surface roughness

**Conclusion** Capacity to realize femtosecond laser endothelial graft precut directly in a storage device could improve the quality and security of delivered lamellar grafts

• 4763

**Integrin alphavbeta5 identifies human corneal epithelial stem cells**

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**Purpose** Stem cell (SC) therapy is the main treatment modality to restore vision in patients with limbal stem cell deficiency. If corneolimbal epithelial stem cells (LESC) can be readily identified, isolated and maintained ex vivo, better quality grafts can be generated for patients with this debilitating disease. With prior knowledge that the extracellular matrix protein vitronectin (VN) is present within the LESC niche and that it supports SC in vitro, we postulated that the VN receptor  $\alpha v\beta 5$  is expressed by, and can be used to identify and isolate SC within the cornea.

**Methods** Epithelial cells were isolate and expand from cadaveric human corneas and  $\alpha v\beta 5$  expression determined by flow cytometry and immunofluorescence. Integrin expressing cells were isolated by magnetic-activated cell sorting then assessed by immunocytology, colony forming efficiency, RT-PCR and microarray analysis.

**Results** Integrin  $\alpha v\beta 5$ + cells co-localized to N-cadherin+/CK-15+ putative LESC.  $\alpha v\beta 5$  was restricted to 3% of the total epithelial cells, which expressed more SC markers and formed more colonies compared to  $\alpha v\beta 5$ - cells. Transcriptional profiling of  $\alpha v\beta 5$ +/- cells by global gene array identified several interferon-inducible genes, including the chemokine IP-10, which localized to LESC. The role of IP-10 in these cells is yet to be elucidated, but may be involved in immunoprotection by attracting mesenchymal SC into the corneal stroma.

**Conclusion** Integrin  $\alpha v\beta 5$  is a candidate SC marker since its expression is restricted and  $\alpha v\beta 5$ + limbal epithelial cells have properties of LESC. Knowledge of the niche's composition and the genes expressed by its SC will facilitate isolation and maintenance of these cells in culture for therapeutic purposes.

• 4762

**Ultrastructural 3D imaging of collagen fibrils and proteoglycans of macular dystrophy cornea**

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**Purpose** Macular cornea dystrophy (MCD) is an autosomal, recessive, genetic disorder, involving a gene on chromosome 6 that leads to aberrant keratan sulphate synthesis. Here we report ultrastructural 3D image analysis of collagen fibrils (CF) and proteoglycans (PGs) macular corneal dystrophy (MCD) corneas.

**Methods** Three normal and three macular dystrophy (MCD) corneas were used for the study. The corneas were fixed in the 2.5% glutaraldehyde containing cuproline blue and processed for electron microscopy. 120 images were taken and used to construct 3D images of CF and PGs.

**Results** 3D image analysis showed that the PGs were embedded within the collagen fibrils of the stroma. There were less electron dense particles within the CF of the MCD cornea compared to within the normal CF. The diameter of the CF in the anterior stroma was larger compared to the CF diameter in the posterior stroma. The PGs area in the anterior stroma was significantly larger compared to the PGs area in the middle and posterior stroma. The PGs around the degenerated keratocytes were much larger. PGs areas in the MCD cornea were significantly larger than the PGs areas of the normal cornea.

**Conclusion** Ultrastructural 3D imaging provides detailed ultrastructural features of CF and PGs. A large aggregation of the PGs in the anterior stroma suggests that the degeneration of the anterior stroma was more severe compared to the degeneration of the middle and posterior stroma.

• 4764

**Predictive value of optical coherence tomography in graft attachment after Descemet membrane endothelial keratoplasty**

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**Purpose** To evaluate the predictive value of early anterior segment optical coherence tomography (AS-OCT) on graft adherence or detachment after Descemet membrane endothelial keratoplasty (DMEK).

**Methods** Retrospective study of prospectively collected data at a tertiary referral center: 87 eyes of 87 patients out of a consecutive series of 142 DMEK surgeries were included in the study AS-OCT was performed within the first hour after DMEK, and at 1-week, 1-month, 3-months and 6-months, and for each time interval detachments were classified as 'none',  $\leq 1/3$  detachment,  $> 1/3$  detachment of the total graft surface area, or 'complete' detachment. Throughout the study no re-bubbling procedures were performed. The main outcome measure was graft adherence at various postoperative time intervals.

**Results** 1-hour AS-OCT scans were more accurate at predicting the final 6-month graft adherence status than those at 1-week or 1-month. Grafts showing complete attachment or  $< 1/3$  detachment at 1-hour, remained stable or improved in 73% of the cases at 1-week, 82% at 1-month, 86% at 3-months, and 90% at 6-months, respectively. All grafts attached at 1-week, remained attached at 6-months. Graft detachments of  $> 1/3$  at 1-hour showed re-attachment at 6 months in 25% of the cases, while 67.5% of the cases showed a persistent detachment of  $> 1/3$  at 6 months and 12.5% a complete detachment.

**Conclusion** The 1-hour AS-OCT showed best predictive value on 6-month graft adherence status. The combined information of the 1-hour and 1-week AS-OCT may facilitate decision making on surgical re-intervention after DMEK.

• 4765

### Filamentary keratitis epidemiology

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**Purpose** To analyze concomitant pathology contributing to filamentary keratitis.

**Methods** We examined 38 patients (76 eyes) with filamentary keratitis. All of them were consulted by co-specialists (internist, endocrinologist, rheumatologist) with the object to reveal concomitant diseases. The treatment regimen included keratoprotectors, artificial tears, 0,1% dexamethasone, 0,05% cyclosporine. Besides, in case of decompensated concomitant disease patients were treated by appropriate specialist. Among investigative methods were: biomicroscopy, Shirmer test – 1, TBUT-test. Follow-up period was 3-5 months.

**Results** Among concomitant pathology we revealed:

Thyroid gland dysfunction (hypothyreosis) – 34,2 %

Rheumatoid arthritis – 21,1 %

Sjögren's Syndrome – 2,6 %

Diabetes mellitus of 2nd type – 7,9 %

Combination of rheumatoid arthritis and thyroid gland dysfunction (nodes against a background of euthyreosis) – 10,5 %

Combination of diabetes mellitus of 2nd type and thyroid gland dysfunction – 13,2 %

Neither endocrine nor rheumatoid diseases revealed – 10,5 %. Among them 1 patient after previous PRK. Shirmer test – 1 results were variable and no appropriateness with treatment regimen was found. TBUT-test data before treatment were low (6-9 sec.) with an increase up to 9-11 sec. after the treatment.

**Conclusion** 89,5 % of patients had endocrine pathology or rheumatoid diseases or their combination. With this in view, we may consider filamentary keratitis not as a form of severe dry eye syndrome, but as a separate dystrophic corneal disorder with dry eye as its symptom.

• 4766

### Cultivation and characterisation of human peripheral cornea derived endothelial cells

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To confirm that human corneal rims left over from DALK/DSEK/PK surgeries could be useful sources for ex vivo endothelial cell expansion. Human corneal rims remaining from DALK/DSEK/PK surgeries were utilized (1:1 sex ratio, age 63+20 years, endothelial cell density >2,500 cells/mm<sup>2</sup>). The time from death to use varied between 3 days and 1.5 months. Endothelial cells isolated using a two-step, peel-and-digest method, whereby the Descemet's membrane and endothelial cells were peeled off under a dissecting microscope, followed by digestion in collagenase. The isolated cells were suspended in TrypLE prior to plating onto FNC-coated tissue culture plates. The cells were then cultured in Ham's F12:M199 (1:1) media supplemented with, ascorbic acid, transferrin, sodium selenite and bFGF. Characterisation of the cultured cells was performed by RT-qPCR and immunofluorescence staining accordingly. The number of isolated endothelial cells was repeatedly low (< 20,000 cells). However, improved techniques allowed to reduce stromal cell contamination. It was observed that endothelial cell proliferation was improved when the culture surface area was reduced. Furthermore, typical endothelial cobble stone morphology was observed when the cell density was high. Cell morphology and growth showed notable difference related to donor age and preservation time. ZO-1, Na/K-ATPase and PITX2 were used to confirm the endothelial phenotype. Preserved human corneal rims can be utilized for ex vivo expansion of corneal endothelial cells but further optimization is needed.

• 4767

### Adeno-associated viral vector serotypes for gene transfer to corneal endothelial cells

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**Purpose** The aim of this study was to determine the efficacy and efficiency of the non-pathogenic recombinant adeno-associated viral vectors (rAAV) for gene transfer to EC. Therefore, different rAAV pseudotypes were compared to assess the expression and kinetic patterns of a reporter gene (GFP).

**Methods** A comparison of GFP expression and kinetics after EC transduction using a AAV 2/1, 2/2, 2/5, 2/8, 2/9, 2/10 was performed on both murine EC (Balb/C) and human EC (corneas, cell line and primary cells). In addition, the effects of different vector concentrations ( $3 \times 10^3$ / $10^4$ ,  $10^5$ ,  $10^6$ ,  $10^7$ ,  $10^8$  IU/ $\mu$ l) were investigated. Analyses were performed using laser scanning microscopy and flow cytometry.

**Results** Significant differences between human and murine EC as well as primary and immortalized EC were detected. Murine corneal EC transduced with AAV2/2, 2/5 and 2/9 resulted in considerable protein expression, AAV 2/5 did not show considerable expression in human corneas. A slow onset of GFP expression both in immortalized and primary EC was detected, leading to peak and stable expression around 2-3 weeks. Peak expression was reached earlier in primary EC and immortalized murine EC compared to immortalized human EC. The overall plateau of expression was highest in primary EC (~80%).

**Conclusion** Pseudotypes of recombinant AAV alter in their tropism and transduction efficiency between murine and human corneas. In general high levels of gene expression can be obtained with several of these pseudotypes. Characteristic slow-onset kinetics of protein expression have to be taken into account when applying AAVs in translational applications, e.g. in corneal storage in eye banks.

## • 4771

**Iron antagonism of DICER1 promotes NLRP3 inflammasome priming due to enhanced Alu RNA stability**

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**Purpose** Excessive free iron induces retinal toxicity in several human diseases and has been linked to age-related macular degeneration. Iron toxicity is widely attributed to its ability to catalyze hydroxyl radical formation through Fenton's reaction. Iron was recently described as an inhibitor of canonical DICER1 enzymatic activity through sequestration of the cofactor poly(C)-binding protein 2 (PCBP2). We sought to determine whether iron similarly impaired non-canonical DICER1 activity to clear cytotoxic Alu RNA in the retinal pigmented epithelium.

**Methods** Iron overload was induced in human ARPE-19 cells by supplementing culture media with ferric ammonium citrate for 72 hours and in wild-type mice by subretinal injection. Alu, B1 and B2 RNA abundance was measured by northern blotting. Alu RNA/DICER1/PCBP2 binding was assessed by immunoprecipitation, western blotting and LC-MS. Alu RNA cleavage efficiency was evaluated using synthetic in vitro transcribed Alu RNA and recombinant human DICER1 and PCBP2. Inflammasome priming was assessed by quantifying NLRP3 mRNA via qRT-PCR.

**Results** Iron overload in cells and mouse retina induces robust accumulation of Alu transcripts as well as the rodent homologs B1 and B2 RNAs. Iron overload impairs Alu RNA clearance by RPE cells, independently of DICER1 mRNA abundance. Alu RNA binds to the iron sensitive co-factor PCBP2, which enhances DICER1-mediated Alu RNA cleavage. Iron overload or siRNAs targeting PCBP2 prime the NLRP3 inflammasome, which can be prevented by antisense-mediated Alu RNA antagonism.

**Conclusion** These data suggest that Alu RNA induced inflammasome signaling could contribute to retinal iron toxicity in addition catalytic free radical generation.

## • 4772

**Quercetin counteracts the cellular damage caused by HNE and inhibits inflammation in ARPE-19 cells**

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**Purpose** Age-related macular degeneration(AMD) is the leading cause of blindness in the western world. And despite extensive research many questions about disease progression and formation still remain unanswered. One of the driving factors of AMD is a chronic inflammatory process, stimulated by life-long exposure to light and oxidative stress. In the current study we try to evaluate the anti-inflammatory properties of Quercetin, a plant derived polyphenol, and to determine the pathways by which it inhibits inflammation.

**Methods** Cultured ARPE-19 cells were treated with the lipid peroxidation endproduct 4-Hydroxyneonal (HNE) to induce an inflammatory response. Quercetin was added 1 hour after stimulation with HNE to assess its power to suppress an already activated inflammatory response. The effects of the treatment on intracellular inflammation were measured with ELISA and quantitative Real-Time PCR. Cell viability was assessed using the lactate dehydrogenase (LDH)-assay.

**Results** Our results show that Quercetin decreased the levels of the pro-inflammatory cytokines MCP-1 and IL-8. It also protected the cells from HNE-induced toxicity, as was evidenced by a decrease in LDH levels. Quercetin lowered the levels of mitogen-activated protein kinase (MAP) p38 and of phospho-CREB but did not affect the levels of NF- $\kappa$ B or the DNA binding efficacy of its subunit p65.

**Conclusion** Our results show that Quercetin can reduce the inflammatory response in retinal pigment epithelial cells by down regulating the MAPK pathway and decreasing the phosphorylation of CREB. Furthermore it is able to protect cells from death induced by oxidative stress. Quercetin may, therefore, be a valuable tool in the therapy of inflammation in AMD.

## • 4773

**In vivo toxicity evaluation of mannitol included in freezed dry PEA-III microparticles**

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**Purpose** To analyze short term toxicity of the mannitol included (3,375 µg; 0,225%) in an unilateral single intravitreal (ITV) injection (1.5µl) of freezed dry polyester amide (PEA) microparticles ("MPs").

**Methods** SD rats were divided into: G1: ITV Mannitol plus BSS; G2: ITV BSS; G3: Aged-matched control. Clinical examination and animal sacrifice were performed 24 hours, 3 and 7 days post-injection (PI). Tissue sections were processed for H&E and immunofluorescence with antibodies against GFAP, Iba-1 and MHC class II.

**Results** No clinical signs of inflammation were observed at any time point of the study. H&E: The conjunctiva in G1 and G2 had an inflammatory infiltrate that peaked at 24 hours and decreased by day 3. On day 7, tissues looked as aged-matched control. In both groups, some inflammatory cells were found in the vitreous 24h and 3 days PI, the latter being more intense in G1. Immunostaining: the reactivity of astrocytes and Müller cells in G1 was higher than in G2 up to day 3. In day 7 PI, there was a mild and sectorial reaction of Müller cells in G1. Microglia in G1 and G2 showed morphological signs of activation 24h and 3 days PI, the reaction being stronger in G1. On day 7 PI: i) all microglia in G2 and the microglia in the outer retina in G1 looked like control; ii) the microglia in the inner retina showed signs of activation in G1. No MHC-II upregulation was found in either group.

**Conclusion** Intravitreal injection of 1.5µL of mannitol (3,375 µg; 0,225%) induces changes in the glial cells of SD rat retina that should be taken into account in biocompatibility assays after intravitreal injections of mannitol freezed dry microparticles.

## • 4774

**Retinal pigment epithelium cell-derived microparticles mediate oxidative stress-induced retinal cells dysfunction**

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**Purpose** Age-related macular degeneration (AMD) represents the leading cause of vision loss in the elderly. The cumulative oxidative injury induces retinal pigment epithelium (RPE) membrane microparticles production, RPE cell death and cellular senescence. The RPE blebs are implicated in the formation of sub-retinal deposit. Nonetheless, the pathophysiological roles of RPE microparticles (RMPs) remain largely unexplored. This study was designed to investigate whether RMPs participate in the retinal cells dysfunction.

**Methods** RMPs and fluorescent Dil-labelled RMPs were isolated from cultured ARPE-19 cells under oxidative stress. RMPs-treated RPE cells were subjected to WST-1, cellular senescent, apoptotic assay and FACS cell cycle analysis respectively. The antibody against CD36 was used in uptake experiment to determine the involvement of scavenger receptor CD36.

**Results** Our study revealed that uptake of RMPs by RPE cells is time-dependent, and this process is partially dependent on CD36 evidenced by an approximately 50% decrease of RMPs uptake caused by CD36 antibody treatment. In addition, RMPs significantly reduced RPE cell viability in a dose-dependent manner. RMPs in a concentration of 5% µg/ml significantly induced RPE cell-cycle arrest at G0/G1 phase. RMPs-treated cells exhibited a 19% increase in G0/G1 phase, with associated increases of the senescence-associated β-galactosidase activity.

**Conclusion** We demonstrated for the first time that RPE cells uptake microparticles derived from RPE cells under oxidative stress. These findings strongly suggest that RMPs function as mediators to exacerbate the oxidative damages to RPE cells, and indicate a pathological role of RMPs in AMD.



# Posters

- Posters T001 - T089, exhibited on Thursday ..... 200
- Posters F001 - F094, exhibited on Friday ..... 223
- Posters S001 - S100, exhibited on Saturday ..... 247

• T001 / 2477

**Comparative gene expression analysis of corneal stroma mesenchymal stem cell-like cells, limbal epithelial stem cells and bone marrow-derived mesenchymal stem cells**

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**Purpose** Purpose Stem cells in the central part of the cornea serve an important regenerative and homeostatic function. We aimed to describe the genetic fingerprint of these cells and compare that to limbal epithelial stem cells (LESC) and bone marrow-derived MSCs (bmMSCs).

**Methods** Corneal tissue and bmMSCs were harvested from cadavers and healthy donors, respectively (according to the Guidelines of the Helsinki Declaration) and cultured ex vivo. MSC-specific cell surface markers- and genome-wide microarray analysis were performed using FACS and Affymetrix GeneChip Human Gene 1.0 ST Array (~23,000 gene transcripts).

**Results** Genes related to stemness (228), differentiation and lineage (220), cell cycle (108) and HOX, SOCS, Notch signaling (218) were collected into functional groups and clustered hierarchically: 45 genes were found to be specific for corneal stroma (CS)-MSCs, 62 for LESCs and 9 for bmMSCs. The hierarchical clustering clearly separated the CS-MSCs from the LESCs and bmMSCs, but formed a higher cluster with the later. The top 10 genes related to the differences were VCAM1, FNDC1, MFAP5, SFRP2, IGF2, MMP, ITGA2, COLEC12, SEMA3A and MGARP. Number of molecules functioning in cellular movement (381), cellular growth and proliferation (408), development (370) and cellular development (360) were found with top biological functions in CSMSCs compared to LESCs or bmMSCs ( $p<0.001$ ).

**Conclusion** Our data show clear distinction between the studied stem cells based upon their gene expression patterns and strengthen the hypothesis that CSMSCs are derived from bmMSCs and not from LESCs.

• T003

**Bafilomycin A1 enhances the production of IL-1 $\beta$  induced by the proteasome inhibitor MG-132 but not that of IL-8**

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**Purpose** Autophagy and proteasomal degradation, two central clearance systems of the cell, decline during aging. Increased amount of protein aggregates and other waste material compromise normal cellular functions. Inflammasomes are intracellular protein complexes which can become activated by diverse danger signals. It has recently been shown that a decline in cellular clearance systems can offer a sufficient signal for the inflammasome activation. In the present study, we have studied the effects of impaired proteasomal degradation and autophagy on inflammation in ARPE-19 cells.

**Methods** In order to inhibit the proteasomal degradation and autophagy, ARPE-19 cells were treated with MG-132 and Bafilomycin A1, respectively. Productions of IL-1 $\beta$  and IL-8 were measured using ELISA method.

**Results** Our data shows that MG-132 increases the productions of IL-1 $\beta$  and IL-8. The addition of Bafilomycin A1 further increases the amount of IL-1 $\beta$  but not that of IL-8.

**Conclusion** Our data suggests that intracellular protein aggregates are capable of inducing inflammation through several pathways. Production of IL-1 $\beta$  indicates the activation of inflammasome signaling. Interestingly, the inflammasome signaling seems to become further enhanced when autophagy is blocked in addition to proteasomes. Meanwhile, the production of IL-8 remains at the same level.

• T002 / 4667

**Proteasome dysfunction in retinal pigment epithelium during aging contributes to the pathogenesis of Age-Macular related Degeneration**

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**Purpose** Age-related Macular Degeneration (AMD) is the leading cause of blindness in people older than 50 in developed countries, and is associated with formation of subretinal deposits (drusen) and damage to Bruch's membrane (BM) basal to the retinal pigment epithelium (RPE). It has been reported that activities of the ubiquitin-proteasome pathway (UPP) in retinal pigment epithelium decrease upon aging. Previous studies demonstrated that expression of ubiquitin in which lysine 6 is replaced by tryptophan (K6W-Ub) impairs the function of UPP. The main objective of this work was to investigate the hypothesis that chronically impairment of UPP in RPE contributes to some of the cardinal features of AMD, including drusens.

**Methods** To address this question ARPE-19 cells were infected with empty vector, K6W Ubiquitin mutant and Wild Type Ubiquitin. The drusen formation and accumulation in basal matrix was evaluated by confocal microscopy, using specific drusen markers such as APOE, while exosome realease was determined by western blot (such as CD63 and Tsg101) and flow cytometry following exosomes isolation by ultracentrifugation.

**Results** The results obtained showed that expression of K6W mutant Ub leads to an impairment of proteasome activity and an increased amount of exosomes release and drusen accumulation.

**Conclusion** In this study the data might provide a vaulable tool to elucidate the biological determinants of age-related proteolytic stress and its impacts on proteostasis in the retina.

• T004

**AICAR promotes cleansing of MG-132-induced protein aggregates in ARPE-19 cells**

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**Purpose** Age-related macular degeneration pathogenesis involves impaired protein degradation in retinal pigment epithelial (RPE) cells. The ubiquitin-proteasome system and the autophagy pathway are major proteolytic processes in eukaryotic cells. SQSTM1/p62 has been shown as a key player linking the proteasomal and lysosomal clearance systems. The present study investigated the effects of AICAR (AICA ribonucleotide, 5-aminoimidazole-4-carboxamide-1- $\beta$ -D-ribofuranoside) with/ without MG-132 (proteasome inhibitor) on autophagy regulation in RPE cells.

**Methods** ARPE-19 cells were treated with MG-132 (5 $\mu$ M) and/or AICAR (2mM). p62 and MAP1LC3A/LC3 (LC3II) were analyzed by Western blotting. LC3 lipidation was used to study autophagic flux. Transmission electron microscopy was used to detect protein aggregates and autophagosomes. pDendra2-hLC3 construct was used to detect macroautophagy in confocal microscopy analysis.

**Results** AICAR+MG-132 co-treatment induces autophagy clearance of p62 and increased LC3 lipidation. AICAR is able to completely abolish the MG-132-induced protein aggregation after 24 h treatment; at the same time, the co-treatment increases the number of autophagic vesicles. Cells treated with MG-132+AICAR exhibit a strong reduction of the perinuclear aggregates containing both LC3II and p62 proteins.

**Conclusion** Autophagy is emerging as a novel target of therapies aimed to counteract protein aggregation and improve cell viability. In this way, our findings indicate that AICAR could be useful in the acceleration of protein clearance in RPE cells.

**• T005****Hypoxia stimulates the release of Brain Natriuretic Peptide (BNP) from RPE cells**

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**Purpose** The natriuretic peptide system (A-, B- and C-type) has a powerful effect on systemic blood circulation (natriuresis, diuresis and plasma shift) causing haemoconcentration causing increased oxygen carrying capacity of blood. A high concentration of a member of the natriuretic peptide family has been found from the vitreous of patients suffering of proliferative diabetic retinopathy (PDR). The stimulus to which the natriuretic peptide system responded in PDR, however, has remained unknown. We tested the hypothesis that hypoxic conditions will increase the release of BNP (B-type of natriuretic peptides) from human retinal pigment epithelium (RPE) cell culture.

**Methods** Human retinal pigment epithelium cells (ARPE-19) were exposed to hypoxia for 2h, 4h, 6h, 8h and 24h. The amount of BNP and VEGF (positive control) were measured at different time points in culture media using EIA. Hypoxia-Inducible Factor (HIF), a protein, which is the master switch in regulating all the responses to hypoxia downstream, was measured using Western blotting.

**Results** At 24h, the amount of BNP in culture media was significantly higher in hypoxic than in normoxic conditions. Also, the concentration of both VEGF and HIF were significantly higher in hypoxia.

**Conclusion** Hypoxia increases the release of BNP from RPE cells and the response is mediated by HIF. The present results characterize for the first time a stimulus for the natriuretic peptide system in human retina and explain previous clinical findings. Thus, the measurement of natriuretic peptides in the vitreous may guide the treatment of the intraocular diseases in which the retina is suffering from hypoxia. Future perspectives: Natriuretic peptides regulate oxygen transport in all tissues?

**• T006****PKC activation affects, via ELAVL1/HuR protein, VEGF expression in pericytic/endothelial coculture**

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**Purpose** To explore whether, following direct contact, there is a mutual influence between pericytes (PC) and endothelial cells (EC), and to establish whether PKC activation, a condition associated with hyperglycaemia in diabetic retinopathy (DR), can affect, via the mRNA-binding ELAVL1/HuR protein, VEGF expression.

**Methods** Retinal PC and EC were cultured separately (MONO) or in direct contact (1:1 ratio; coculture; COCU), and exposed or not to PKC activator phorbol esters (100 nM PMA, 15 min.). PKC $\beta$ I/ $\beta$ II, HuR, VEGF proteins were analysed by Western blotting; VEGF secretion in cell culture medium was evaluated by ELISA. VEGF mRNA analysis was performed by real-time qPCR. Immunocytochemistry was performed to evaluate PMA-induced HuR translocation.

**Results** In MONO, VEGF mRNA/protein basal levels are higher in PC than EC. In COCU, VEGF protein amount is up-regulated in EC and down-regulated in PC, respectively. VEGF release is favoured by physical contact between PC and EC and further increased by PMA exposure. PKC $\beta$  activation induces HuR translocation from nucleus to cytoplasm, and it increases the protein levels of the kinase itself, HuR and VEGF in PC and EC in both culture conditions.

**Conclusion** VEGF expression varies in function of the cell culture conditions. In all cultures, PKC activation increases PKC $\beta$ I/ $\beta$ II, HuR and VEGF protein levels, changes which may occur also at very early stages of DR. The COCU model may be useful to study PC/EC interactions in both physiological and pathological conditions.

**• T007 / 4666****The effect of hyperglycaemia on permeability and tight junction components in human retinal and choroidal endothelial cells**

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**Purpose** Diabetic retinopathy is the leading cause of preventable blindness in the working population. The main cause of visual loss in diabetic retinopathy is diabetic macular oedema caused by an increase in microvascular endothelial permeability. The aim of this project was to determine the effect of hyperglycaemia, *in vitro*, on human choroidal (hCEC) and retinal microvascular endothelial cells (hREC) with hyperglycaemia.

**Methods** Microvascular permeability was assessed through passage of dye through a confluent cell layer in the presence of hyperglycaemia. Microarray analysis and western blotting was used to compare the expression of selected tight junction molecules (Occludin, Claudin-5, JAM-A and JAM-C) and adheren junction (VE-Cadherin) molecules was compared between hCEC and hREC and with hyperglycaemia.

**Results** Hyperglycaemic conditions significantly increased the permeability in both hCEC and hREC. Microarray analysis and western blotting determined that the baseline hREC expression of occludin and claudin-5 was higher than hCEC. In hREC exposed to hyperglycaemia claudin-5, occludin and JAM-A were found to be reduced. None of the proteins were decreased by hyperglycaemia in hCEC.

**Conclusion** Although hyperglycaemia increased permeability in both hCEC and hREC, tight junction protein expression was only reduced in hREC, indicating a different mechanism of increased permeability in hCEC compared to hREC.

**Commercial interest****• T008 / 2476****Glucocorticoid modulation of agonist induced microvascular endothelial permeability**

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**Purpose** Conditions such as diabetic retinopathy and neural inflammation involve microvascular barrier breakdown leading to leakage, tissue oedema and immune cell influx. Glucocorticoids(GCs) have been shown to improve the barrier function of the vasculature in retina and brain. It has been proposed that GCs function through tightening of specialized junctions between endothelial cells (ECs) and reduce paracellular permeability. We investigated the effects of hydrocortisone(HC),dexamethasone(DEX),triamcinolone(TA) and a selective glucocorticoid receptor agonist(SEGRA) on microvascular endothelial permeability induced by vascular endothelial growth factor(VEGF),lysophosphatidic acid(LPA) and histamine(Hist), all of which are proposed to be involved in diabetic and inflammatory neurovascular pathologies.

**Methods** *In vitro* studies were undertaken in cultures of primary rat brain and retinal microvascular ECs, as well as a novel immortalised rat retinal EC line, PT2. Junctional protein characterisation was performed by indirect immunocytochemistry and confocal microscopy. Permeability was measured by macromolecular flux assays.

**Results** Primary retinal and cerebral EC cultures exhibited an exquisite apico-basal polarity in their response to vasoactive compounds, maintained high barrier properties and sophisticated junctional protein complement. Passaged or immortalised EC had lost most of these features. HC and DEX were effective in suppressing VEGF-, LPA- and Hist-induced permeability. TA results were similar, except Hist-induced permeability was insensitive to TA. SEGRA was ineffective in preserving microvascular barrier function.

**Conclusion** The effectiveness of GCs depends on the pathology involved in particular the vasoactive substance in play.

**• T009****Macro and microglial retinal cells in rat organotypic cultures**

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**Purpose** Organotypic retinal cultures constitute a useful tool to perform preclinical drug testing. The aim of the present study was characterize the morphologic changes in macro- and microglial cells in this culture system.

**Methods** Retinas were isolated from 7-day-old Crl:CD(SD) rats with the retinal pigment epithelium attached. Retinal explants were cultured for 4, 10, and 14 days (DIV4, DIV10, and DIV14). Cryosections and whole-mounts of age-matched control and cultured retinas were used to analyze macro- and microglial cells by immunostaining using antibodies against GFAP, vimentin and CD11-b.

**Results** In comparison with *in vivo*, in DIV4 and DIV10 cultures, GFAP-positive astrocytes were more robust, the astrocytic network being thicker in some retinal areas while in other regions astrocytes were sparsely distributed. Few thin GFAP-positive astrocytes were observed at DIV14. Müller cells in the cultures exhibited GFAP up-regulation in comparison with *in vivo* retinas. CD11-b+ microglial cells at DIV4 and DIV10 showed more robust somas and thicker and more retracted processes than *in vivo*. Overall, at DIV14 CD11-b+, microglial cells exhibited a rounded morphology.

**Conclusion** In rat organotypic culture both macro- and microglial cells showed progressive changes: i) reactive macrogliosis, ii) rearranged astrocytic distribution, iii) GFAP up-regulation in Müller cells, and iv) microglial activation. Given that this glial response is a hallmark of several retinal diseases, organotypic retinal culture is a valuable resource for future investigations in retinal degenerative processes and therapy.

**• T010****A new automatic method for microglial-cell quantification in whole-mount mouse retinas**

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**Purpose** To study the proliferative microglial behavior in a laser-induced ocular hypertension (OHT) model with an automatic method that allows the assessment of the number of microglial cells and comparisons of the results with those found by direct human observation.

**Methods** Albino Swiss mice were divided into: age-matched control, n=6; and lasered, n= 6. Retinal whole-mounts were immunostained with anti-Iba1. In Matlab, new algorithms of segmentation and control of distances were developed to determine the number of Iba-1+ cells. The automatic results were compared with those from direct human observation of the images.

**Results** The algorithm method automatically detected the number of Iba-1+ retinal cells in the inner and outer plexiform layers, both in naïve and OHT retinas. The number of cells present in the image samples was not an obstacle for the program to run properly. The time required for counting Iba 1+ cells decreased from the human guide to the program-based counting method from days to one hour. the results showed a strong correlation between automatic and manual methods (Pearson correlation test, R = 0.979, P=0.000 and R= 0.942; P=0.000 for outer and inner plexiform layer, respectively) indicating the reliability of the automatic counting.

**Conclusion** A new consistent and fast algorithm method was developed with Matlab to quantify Iba-1+ microglial cells as well as cellular density maps through retinal whole-mounts, in both naïve and OHT. Through this new automatic method, a larger set of images or samples could be included in future studies to analyze the behavior of microglial cells under proliferative conditions.

**• T011****Characterisation of diabetic retinal neuropathy in Ins2Akita mice**

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**Purpose** To characterize diabetic retinal neuropathy in the Ins2Akita mouse.

**Methods** Male heterozygous Ins2Akita mice (2-8 months of hyperglycemia) and C57BL/6 age-matched siblings were used in this study. Retinal function was assessed by electroretinography (ERG) and thickness measured by optical coherence tomography. Eyes were then processed for immunostaining of different neuronal cells, including photoreceptors, bipolar, horizontal, amacrine and ganglion cells.

**Results** Ins2Akita mice exhibited altered scotopic ERG after 6-8 months of hyperglycemia. The reduction of outer and inner retinal thickness was observed after 2 months of diabetes. Despite no striking abnormalities were found in photoreceptors, a 40% reduction of photoreceptor synaptic ribbons was observed in Ins2Akita mice compared to age-matched controls. This was accompanied by total depletion of horizontal cell dendritic plexus and disarrangement of rod and cone bipolar cell processes at the outer plexiform layer. In the inner retina, the number of rod and cone bipolar cells remained similar to controls, but abnormal GABAergic amacrine cell stratification and 25% reduction of rod bipolar cell axon terminals were observed. Brn3a staining revealed a 20% loss of retinal ganglion cells, which exhibited abnormal aggregates of light neurofilament. In addition, a significant retinal vascular degeneration evidenced by around 50% reductions of vascular plexus was observed in 8 month hyperglycemic Ins2Akita mice. No evidence of retinal neovascularisation was detected.

**Conclusion** Ins2Akita mice present a variety of retinal neuropathies including the reduction in different types of neuronal cells and disruption of synaptic structures. The mice also develop severe retinal vascular degeneration but no neovascularisation.

**• T012****Müller cell response during degenerative retinopathy in mice**

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**Purpose** Structural retinal alterations were evaluated in a murine model of retinopathy with photoreceptor degeneration, in order to establish response patterns for each stage of the inflammatory process.

**Methods** In this work, a murine model of retinopathy with photoreceptor degeneration was studied. Groups of 4 animals were intraperitoneally injected with 100 mg/Kg of sodium iodate, and euthanized 24, 48 and 72 hours after injection. A non-injected animal was used as a control in each group. The retinas were analyzed by means of histological techniques, immunohistochemistry, Western Blotting and q-RT-PCR.

**Results** In the retina, aggressive stimuli are known to induce reactive gliosis. In our murine model of retinopathy, Müller cell activation was accompanied by cell proliferation and glial fibrillar acidic protein (GFAP) over-expression with maximum values 48h after injection.

**Conclusion** The differences in GFAP expression allowed us to establish expression patterns of this protein which may be used to identify the phase of the inflammatory response in progress.

**• T013****Cellular senescence is increased in human retinal microaneurysms during aging**

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**Purpose** Different studies indicates that the presence of retinal microaneurysms, dilations of the capillaries which often appear as gross outpouchings of the vessel wall, increases during human aging. However little is known about the mechanisms that may contribute to the development of these structures. The aim of this study was to examine whether cellular senescence may contribute to the formation of microaneurysms and specifically examine the role of p16INK4a, a cyclin dependent kinase inhibitor, that may act promoting the irreversible cell cycle arrest and senescence.

**Methods** Human retinas were obtained from 14 old-donors and 3 middle-aged donors. p16INK4a expression was analyzed using immunohistochemistry and laser-confocal microscopy. Furthermore, in the same retinas we assessed senescence-associated  $\beta$ -galactosidase (SA- $\beta$ -gal) activity, a widely used biomarker for cellular senescence.

**Results** Microaneurysms were present in all the retinas obtained from old donors but were absent in those obtained from middle-aged donors. p16INK4a expression was strongly increased in all retinal layers of old retinas when compared with middle-aged retinas. We observed that p16INK4a expression was increased in retinal blood vessels of aged donors, in which we also detected the presence of high levels of SA- $\beta$ -gal activity. Furthermore, p16INK4a expression was strikingly increased in retinal microaneurysms when compared with that observed in their associated capillaries.

**Conclusion** p16INK4a was overexpressed in the retinal microaneurysms of aging people indicating that cellular senescence could be a crucial mechanism contributing to the formation of retinal microaneurysms during aging.

**• T015****Evaluation of central retinal thickness and subfoveal choroidal thickness in normal eyes**

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**Purpose** To determine central retinal thickness and subfoveal choroidal thickness using optical coherence tomography (OCT) and to evaluate association between both thickness and age, gender and axial length.

**Methods** Spectral domain OCT images (Bioptrigen, Bioptrigen, inc., Durham, NC) were obtained in 151 eyes of 151 healthy volunteers without retinal diseases. The IOL master (Carl Zeiss Meditech) was used to measure the eye length to compensate axial scale factor of the SD-OCT images. The choroidal thickness at the fovea and central retinal thickness was measured. Statistical analysis using paired t-test and Pearson correlation were performed to evaluate the correlation between both thickness and age, axial length, and gender.

**Results** The mean and median age of the 151 subjects were 33.3 and 29 years old, respectively (range, 7 to 80 years). There were 57 males and 94 females. The mean and median axial lengths of the eye were  $24.54 \pm 0.98$  mm and 24.37 mm respectively. The mean central retinal thickness were  $213.89 \pm 14.64$   $\mu$ m. Increasing age and axial length were not correlated with central retinal thickness. The mean choroidal thickness in these normal eyes was  $214.43 \pm 13.63$   $\mu$ m. Increasing age (Pearson correlation - 0.193,  $R^2=0.0372$ ,  $p=0.018$ ) and increasing axial length (Pearson correlation - 0.189,  $R^2=0.0359$ ,  $p=0.020$ ) were correlated with decreasing choroidal thickness. Central retinal thickness and subfoveal choroidal thickness have no relationship between them in our study (Pearson correlation - 0.139,  $R^2=0.0192$ ,  $p=0.090$ ).

**Conclusion** Only the choroidal thickness at the fovea in normal eyes showed an inverse correlation with age and axial length. And there is no significant relationship between central retinal thickness and subfoveal choroidal thickness.

**• T014****The fractal properties of the retinal vascular architecture**

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**Purpose** The human retinal vasculature has been demonstrated to exhibit fractal, or statistically self similar properties. Fractal analysis offers a simple quantitative method to characterise the complexity of the branching vessel network in the retina. Several methods have been proposed to quantify the fractal properties of the retina.

**Methods** Twenty five healthy volunteers underwent retinal photography, retinal oximetry and ocular biometry. A robust method to evaluate the fractal properties of the retinal vessels is proposed; it consists of manual vessel segmentation and box counting of 50 degree retinal photographs centred on the fovea.

**Results** Data is presented on the associations between the fractal properties of the retinal vessels and various functional properties of the retina.

**Conclusion** Fractal properties of the retina could offer a promising tool to assess the risk and prognostic factors that define retinal disease. Outstanding efforts surround the need to adopt a standardised protocol for assessing the fractal properties of the retina, and further demonstrate its association with disease processes.

**• T016****Assessment of macular retinal thickness and volume in normal eyes and highly myopic eyes with spectral optical coherence tomography**

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**Purpose** To compare the macular retinal thickness and macular volume between subjects with high myopia and non-myopia

**Methods** A prospective comparative study recruited highly myopic eyes with no posterior abnormalities (spherical equivalence (SE) over -6 dioptres (D) or AXL>or=26.5 mm) and subjects with non-myopia (SE between 1.5D and -1.5 D). Macular retinal thickness and volume thickness at the fovea 1.5 mm superiorly, inferiorly, nasally, and temporally were measured with spectral-domain optical coherence tomography (TOPCON SD 2000).

**Results** One hundred and twelve myopic eyes and 66 non-myopic eyes were included. The mean age of the high myopic group and non-myopia group was 34.28 and 28.5 years old, respectively. The mean refraction was -13.98 D in the high myopia group and -0.24 D in the non-myopia group. The high myopia group had significantly greater mean retinal thickness in the foveola  $188.8 \pm 155.2$  microm,  $P<0.0001$ ), and fovea 1 mm area ( $205.8 \pm 181.4$  microm,  $P=0.0088$ ), than the non-myopia group. The mean retinal thickness in the inner and outer macular area of the high myopia group was significantly less than in the non-myopia group. In addition, the high myopia group had significantly smaller macular volume than the non-myopia group ( $P<0.0001$ ).

**Conclusion** The retinal thickness in high myopic eyes is thicker in the foveola and fovea, but thinner in the inner and the outer macular region. The retina of individuals with high myopia had smaller macular volume than those with non-myopia.

• T017

**Macula of retina - spherical calotte with its focus. Right image on the retina. Applications: Biophotonics Laser Holographic Theory of Vision. Apparatus for the study of human vision**

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**Purpose** The paper presents the results of theoretical multidisciplinary studies, whose purpose was to study the biophysical mechanism of vision, but taking in account macula's form as spherical calotte and its own focus, the structure and functioning of the human eye as a bifocal laser system (cornea and macula focus) with holographic elements, to establish the holographic biophotonic mechanism of vision according to the Biological Lasers Theory, to verify the hypothesis using the bionic method.

**Methods** For experimental method we built two optical devices, which to be in line with the latest anatomical data of the eye, at 5:1 and 10:1 scale, according to laser holographic biophotonic model of the human eye: optic monocular apparatus or bionic eye- compact model and scaffold type, which are models of bifocal laser system with intermediate holographic system.

**Results** The results and discussions of studies offer great surprises. The image at macula pole may be directly observed, as a right position, colored, dynamic, much diminished and it may be transmitted from one pole to the other, but has a sense daytime and an inverted sense nighttime, as dreams images. The two optic apparatus confirm the laser holographic biophotonic mechanism of human vision.

**Conclusion** The conclusions of the theoretical and bionic method studies are that Vision Photographic Theory is moral and technical outdated. The Vision Holographic Biophotonic Theory VHBT is modern and according to the principles of lasers, holography, liquid crystals, the nonlinear optics, electronics, biophotonic and human eye physiology.

**Commercial interest**

• T018

**Biological lasers theory, vol.II vision holographic theory - Eye and vision**

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**Purpose** The romanian book deals with the Biological Laser Theory BLT and its applications - the Vision Holographic Theory VHT. The book has about 465 pages, 7 chapters, and 151 figures (85 original figures), 15 tables. After introduction of the actual problems in ophthalmology, are presented: the purpose of the book - to verify some old theory about an up-down image on retina, to demonstrate by theoretical and experimental studies that human eye structure is a bifocal laser system with holographic system included, the bio luminescence is a biological laser phenomena, laser holographic biophotonic mechanism of the sight, and applications;

**Methods** Theoretic method: a short history of the sight mechanism. Our old studies on characteristics of cell biological laser systems; the VHT hypothesis about eye and vision, the coherent transformation of light in bio luminescence, as laser phenomena; multidisciplinary arguments for laser-holographic structures and function hypothesis are from ophthalmology, lasers, holography, liquid crystals, electronics, biophotonic and bionics. As experimental method we present two original apparatus for human vision study acknowledged at EuroInvent Iasi 2011, and CIGIG 2012.

**Results** Both apparatus proves the right position of holograms on retina, due to eye, not to brain. We describe some other holographic-biophotonic mechanism for biological (muscle contraction) and psychic processes (vision, memory, thinking, speech etc), and increasing the power and information along nervous system.

**Conclusion** Important applications are in medicine teaching, physics and bionics. Keywords: eye, bioluminescence, bifocal laser system, holography, image, nervous system, biophotonic mechanism, biologic process, psychic process

**Commercial interest**

• T019

**The activity of the "when" pathway during visual motion**

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**Purpose** Recently, a third pathway, the "when pathway" has been described (Battelli, 2007). The when pathway is project from the Primary Visual Cortex (V1) to the inferior parietal lobe (IPL) through the middle temporal and middle superior temporal (MT+) areas. The activity of the when pathway has not been confirmed experimentally. In this study, the activity of the when pathway was examined during visual motion.

**Methods** A total of 6 healthy volunteers were studied. Task 1 comprised the visual presentation of one black dot alternating with a frequency of 1 Hz. Task 2 comprised the visual presentation of two black dots alternating with a frequency of 1 Hz. Task 3 comprised the visual presentation of three black dots alternating with a frequency of 1 Hz. Electroencephalography (EEG) data were digitized from 32 electrodes, which were placed according to the extended International 10\_20 system. The EEG data at rest and for each task included O2ch between 30\_50 ms in the right primary visual cortex area, P8 between 170\_190 ms in right MT+ areas, and P4 between 200\_220 ms in the right IPL area, and the waveform extracts were performed with power spectrum analyses. The calculated power value in the range of 8\_13 Hz ( $\alpha$  frequency band) from each waveform was examined in V1, MT+, and IPL and compared between rest in each task.

**Results** The  $\alpha$  bands in V1, MT+, and IPL in each task were significantly lower than those at rest ( $p<0.05$ ).

**Conclusion** The crucial function of the when pathway is to keep track of multiple objects that temporally overlap or occur sequentially. In this study, the  $\alpha$  bands in each task were significantly lower compared with those at rest. However, significant differences in the  $\alpha$  bands were not observed in each task. We suggest that the when pathway is active in response to only one object.

• T020

**Evaluation of direct and consensual accommodation of fellow eyes using binocular open field auto refracto/keratometer WR-5100K**

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**Purpose** To develop a method of objective simultaneous evaluation of direct accommodation (DA) and consensual accommodation (CA) of paired eyes.

**Methods** To assess DA and CA, eyes of 83 patients aged 7-25 with different refraction errors were examined. Objective accommodation response was measured using Binocular Open Field Auto Refracto/Keratometer WR-5100K (Grand Seiko Co., Ltd., Japan) under full correction of the existing ametropia achieved by spherical and cylinder lenses in a trial frame. A special device was used to separate the visual fields of the two eyes so that a 33 cm target could only be seen with one eye, while the fellow eye was looking at the open field with no fixation mark. In this way the DA was measured for the fixating eye and the CA for the non-fixating eye. Then the fixation mark position was switched over and the test was repeated. The difference ( $\Delta$ =CA - DA) between CA and DA was calculated.

**Results** In all cases of hyperopia and emmetropia, consensual accommodation was equal to direct accommodation ( $\Delta=0.02\pm0.03$  D and  $0.06\pm0.02$  D, respectively). In low and moderate myopia, an insignificant difference was noted between CA and DA (respectively  $0.16\pm0.04$  D and  $0.23\pm0.03$  D). In high and anisometropic myopia, the difference was significant:  $\Delta=0.27\pm0.04$  D and  $0.48\pm0.07$  D, respectively ( $p<0.05$ ).

**Conclusion** CA and DA did not differ in patients with hyperopia and emmetropia. Mismatch of DA and CA was found in high and anisometropic myopia. This symptom may have diagnostic and prognostic value for progressive myopia.

**• T021****The relationship between halo size and intraocular light scatter in normal healthy subjects**

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**Purpose** To determine the relationship between the size of a halo in the visual field induced by bright light and the intraocular light scatter in normal healthy eyes.

**Methods** Measurements were made in the right eyes of 66 healthy subjects (mean age  $33 \pm 9$  years). Using the Vision monitor, optotypes of low luminance ( $1 \text{ cd/m}^2$ ) were presented at a distance of 2.5 m. The visual angle subtended by the radius of the halo was calculated in minutes of arc (arc min). Retinal straylight was measured with the compensation comparison technique. Best-corrected distance visual acuity (BCVA) was evaluated with logMAR Bailey-Lovie letter charts.

**Results** The mean radius of the halo was  $199.3 \pm 45.6$  arc min, mean retinal straylight was  $0.98 \pm 0.13$  log units and mean BCVA was  $-0.02 \pm 0.07$  logMAR. There was a significant positive correlation between halo radius and straylight ( $r = 0.32, p < 0.01$ ) and between halo radius and BCVA ( $r = 0.36, p < 0.01$ ). However, no significant relationship was found between retinal straylight and BCVA.

**Conclusion** Higher intraocular light scatter levels were related to larger halo size in healthy human eyes.

**• T022****Simultaneous VEP and EOG in dyslexic children**

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**Purpose** We used Pattern visual evoked potential (PVEP) and Electrooculogram (EOG) to clarify the conflicting evidence of neurophysiological abnormalities in dyslexic children.

**Methods** PVEP and EOG were recorded simultaneously in 72 children including 36 dyslexic and 36 normal children that were matched for age, sex and intelligence. Two check sizes of 60 and 15 min arc were used with temporal frequencies of 1.5 Hz for transient and 6 Hz for steady state methods.

**Results** Our findings show no differences in VEP components between dyslexia and normal that did not support the magnocellular hypothesis. High contrast EOG stimulus in detection of dyslexia is more reliable than VEPs.

**Conclusion** The results are not consistent with the evidence of an isolated deficit of the magnocellular function. The high contrast stimulus used in this study is thought to be involved to parvocellular system.

**• T023****Retinal vessel oxygen saturation in retinitis pigmentosa patients**

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**Purpose** To study the retinal oxygen saturation in patients with retinitis pigmentosa (RP).

**Methods** Retinal Vessel Oximetry was performed with the oximetry tool of Retinal Vessel Analyzer (Imedos GmbH, Jena, Germany) on 12 eyes of 6 RP patients and on 24 eyes of 12 age-matched healthy controls ( $p=0.747$ , two-way ANOVA). RP patients were selected following a clinical and electrophysiological assessment. We evaluated the mean oxygen saturation of arteries (A-SO<sub>2</sub>), the mean oxygen saturation of veins (V-SO<sub>2</sub>), as well as the A-V-SO<sub>2</sub> difference and compared them to the ERG values within both groups.

**Results** In controls, the mean A-SO<sub>2</sub> and V-SO<sub>2</sub> of the retina was 92.41% (SD,  $\pm 3.54$ ) and at 53.99% (SD,  $\pm 2.95$ ), respectively. In the RP group, the A-SO<sub>2</sub> and the V-SO<sub>2</sub> were increased to 100.13% (SD,  $\pm 7.24$ ) and 66.96% (SD,  $\pm 5.67$ ), respectively (p-values=0.000). The A-V-SO<sub>2</sub> difference, known to be proportional to oxygen consumption, was reduced to 33.17% (SD,  $\pm 5.85$ ) in RP when compared to the controls 38.39% (SD,  $\pm 3.00$ ) in a linear regression model ( $p=0.001$ ). The b-wave of the dark-adapted 3.0 ERG response compared to A-SO<sub>2</sub>, V-SO<sub>2</sub>, as well as A- V-SO<sub>2</sub> difference within both groups, showed a linear correlation of:  $r=-0.571$ ,  $r=-0.846$ , and  $r=-0.531$ , respectively with the corresponding p-values  $<0.003$ . The b-wave of the light-adapted 3.0 ERG response compared to A-SO<sub>2</sub>, V-SO<sub>2</sub> and A- V-SO<sub>2</sub> difference, showed a linear correlation of  $r=-0.581$ ,  $r=-0.807$ , and  $r=0.472$ , respectively, with the p-values  $<0.008$ .

**Conclusion** This is to our knowledge the first study data, which suggests the oxygen metabolism to be altered in cases with retinitis pigmentosa.

**• T024****Hyperstereopsis is attenuated by the addition of secondary visual cues**

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**Purpose** Telestereoscopic (or hyperstereo-) viewing which increases effective interocular distance has been a subject of interest since Helmholtz's pioneering work. Increased disparities and vergence alter 3D-space perception. In particular, apparent egocentric distances decrease, owing to the increased vergence (Priot et al., 2010; Rogers, 2011). This study sought to investigate how the enrichment of stimulus information influences its perceived distance.

**Methods** We assessed distance perception through open-loop pointing before and during exposure to a telestereoscope in three stimulus viewing conditions: i) a red pinpoint of light (pinhole) providing vergence cue only; ii) a red cross with randomized diameter preventing size cue, providing vergence and some accommodative cues and iii) a real-size coin slide providing vergence, accommodation, relative- and familiar-size cues.

**Results** Before exposure (normal viewing), we observed an improved perceived-distance accuracy induced by enriched stimulus information. During exposure (with vergence discrepancy), we found that, on average: i) telestereoscopic viewing altered perceived distance; ii) this effect was modulated by the stimulus viewing condition; iii) the mean slope of the perceived distance of the cross stimulus vs. its physical distance was not different from that predicted by vergence changes according to Helmholtz's scaling theory; and iv) the hyperstereo effect was attenuated by the enriched stimulus information.

**Conclusion** We ascribe the attenuation of hyperstereopsis by the addition of secondary visual cues to a down-weighting of vergence contribution relative to those of the other cues.

• T025

**Analysis of retinal and cortical response to electrical stimulation by subretinal implant in rodent**

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**Purpose** This aims to develop and improve the use of retinal prosthesis in animal modelFor that purpose we measured retinal and cortical response to direct subretinal electrical stimulation to understand how the patterns of stimuli can be adapted to improve stimulation to get closer to the response evoked by natural visual stimuli

**Methods** In a rat model, a comparative analysis of the functional impact of similar stimulation of a subretinal implant is done at two levels (1)in the retina in vitro by multi electrodes array (2)in vivo in the primary visual cortex by optical imaging recordingsOptical imaging permits a functional mapping of the cortex, using light reflection and absorption changes depending on the rate of blood oxygenationDiverse parameters were investigated: stimulus shape and polarity/intensity/size and location

**Results** At the cortical level we have quantified the size, position and intensity of the point-spread function in response to the various electrical stimulations and compared them to those generated by calibrated light stimuli. The point-spread function was much larger for electrical stimulations compared to visual stimulationWe have performed retinal recordings of ganglion cells, while the prosthesis is on the photoreceptor sideThe cortical response and the recruitment of ganglion cells respond according to a logarithmic equationThe ability to evaluate in vitro within the retina and in vivo the cortical responses induced by the prosthesis allowed us refining the patterns of electrical stimulation to get closer to a natural activation

**Conclusion** These results offer interesting prospect for improving the design of prostheses as well as their patterns of stimulation for a medical application

• T026

**VEP evidence of significant differences in motion perception in children**

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**Purpose** In adults the motion-onset visual evoked potentials (M-VEPs) represent a useful diagnostic tool in neuro-ophthalmology (Kuba et al., Vision Res., 2007, 47, 189-202) since they test quite selectively the magnocellular system/dorsal stream function. Whilst in adults robust M-VEPs with low variability can be acquired easily, it is difficult to record and evaluate them in children. They not only display very long maturation and age dependent shape development (Langrova et al., Vision Res., 2006, 46, 536-544) but in some children they are unidentifiable to standard stimuli.

**Methods** To understand better this problem we tested 30 normal children aged 7 - 12 years. Two types of motion stimuli were used – translation motion (TM) and expansion/contraction motion (EXCOM).

**Results** Reliable VEPs were detected in 77% of children to TM and in 83% of children to EXCOM. The dominant peak of the VEPs was negative in all TM VEPs and in 80 % of EXCOM VEPs (positive in 20%). In both VEP variants the latency of the negative peak did not shorten significantly in the tested age range and there was large interindividual latency variability (176 ms – 268 ms). High contrast stimuli (non-optimal "magnocellular" stimulus) shortened the M-VEPs latencies and enlarged amplitudes in majority of subjects. M-VEPs to peripheral stimulation (outside central 20°) were in contrast to adults detectable only in 43 % of children.

**Conclusion** These findings show distinct differences in maturation of motion perception in children, which makes a diagnostic use of M-VEPs in children quite complicated. Acknowledgement: Supported by the project PRVOUK - P37/07.

• T027

**Effects of Quensyl on the ERG a-wave amplitude from the isolated superfused vertebrate retina**

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**Purpose** Long-term therapy with quensyl is known to cause neurodegenerative changes in the retina. In our present research we study acute toxic effects of quensyl on the a-wave response of electroretinogram (ERG) of isolated superfused bovine retinas.

**Methods** Isolated bovine retinae were mounted in a temperature-controlled recording chamber. After light stimulation electric field potentials were recorded as a transretinal potential using Ag/AgCl-electrodes. Isolated bovine retinas were perfused with phosphate buffered saline (PBS) containing 1mM L-aspartate to block further synaptic transmission in order to record the effects of quensyl on photoreceptors. We tested the low and high light intensities of 100 mlux and 10 lux. After reaching a stable ERG amplitude, quensyl (190 µM, 570 µM or 1.9 mM) was added to the perfusing solution. After 90 min quensyl was washed out for 90 min with PBS containing 1mM L-aspartate. Changes in a-wave amplitude were calculated and plotted.

**Results** 190 µM quensyl showed a 1.3 fold stimulation of the a-wave amplitude at 100 mlux, the effect at 10 lux was not significant. 570 µM quensyl reduced the a-wave amplitude by 3-folds independent of light-intensity. The inhibition was good reversible by washing with PBS containing 1mM L-aspartate only at low light intensity (2 folds), there was almost no recovery at 10 lux. 1.9 mM quensyl showed a massive depression of a-wave amplitude (6 to 7 folds) and no wash out effect over 90 minutes at both light intensities.

**Conclusion** Quensyl has a toxic effect on photoreceptors, even with slight increase of concentration showing a huge progression of inhibition and reduction of recovery. An exact dosage of quensyl is of great importance to avoid an irreversible neuronal damage.

• T028

**Keep an eye on the Pi – Using the Raspberry Pi as inexpensive, yet powerful platform for vision research**

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**Purpose** In vision research as well as in other research disciplines, like psychology or psychophysiology, there is often a need for presenting visual stimuli and for registration of a physiological responses, like the pupil diameter, to them. Commonly, this is realized by making use of notebooks or desktop computers, therefore, wasting resources and energy. Here, we present a new platform for vision research based on the Raspberry Pi (RPI), an inexpensive, yet powerful system-on-a-chip (SoC).

**Methods** The ARM based RPi, running up to 1GHz with 512MB RAM and offering interfaces like HDMI, RJ-45, USB and GPIO at a price of \$35 only, is a convenient basis for vision research. We took a recently published study dealing with pupil responses to pictures of light sources as draft to test its suitability for this usage. One RPi was used for controlling stimulus presentation, a second one for measuring the pupil diameter based on a video stream. The software was implemented using Java on a debian linux.

**Results** Due to the limited memory and processor performance, the framerate was restricted to about 20fps. However, it allowed for the recording of changes in the pupil diameter in response to the presented stimuli. We successfully comprehended a previous study, and thus showed that the RPi is a serious and cheap alternative to using notebooks or desktop computers for vision research.

**Conclusion** In spite of its size, the RPi provides surprising high performance. Based on open-source software, applications can be implemented in languages like Java or Python, leveraging existing software packages. After the advent of the Raspberry Pi, similar devices became available, providing even more performance for low prices.

• T029

**Differential diagnosis of endogenous visual phenomena**

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**Purpose** To draw attention to either goal-targeted or misleading features of phantom images originating from the optical media of the eye, retina, visual pathways and cortex.

**Methods** Retrospective analysis of cases, connecting patient reports on various patterns of endogenous visual phenomena with pathology at different levels of the visual system, by keratotopography, refractometry, perimetry, biomicroscopy, OCT and electrophysiology.

**Results** Common features of our case histories are patient reports on visual phenomena which lack clear correlates to the outside visual environment. We demonstrate how the above diagnostic tools discern optical photic phenomena from mechanical phosphenes, vitreous traction phosphenes from photopsias arising due to retinal pathology and from lightning due to optic neuropathy. Criteria are defined for discerning these altogether from the various appearances of filling-in phenomena in visual field scotomas.

**Conclusion** A thorough interpretation of a patient's report on endogenous visual phenomena provides a valuable starter into ophthalmologic diagnosing and into some better understanding of disease mechanisms.

• T030

**Upper hemiretinal occult retinopathy**

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**Purpose** To emphasize the significance of photopsias and positive scotomas as subjective indicators of occult retinopathy.

**Methods** The patient's depiction of his photopsias led to a diagnostic work-up by white and red static and kinetic perimetry, OCT, and visual electrophysiology.

**Results** Within the OD lower visual field our patient encountered a rapid loss of color vision. In dark environs, coloured photopsias showed up in this region, in bright environs, a positive black scotoma developed over time. Kinetic perimetry revealed a relative lower hemifield scotoma by white and an absolute one by red targets. On static perimetry, a distinct lower hemifield scotoma improved for white but persisted for red stimuli during 3 years of follow up. Retinal origin of the scotoma was proven by mf ERG. Serology for anti-retinal antibodies remained negative. Difficult to detect by ophthalmoscopy, an affection of the OS / RPE layer in the corresponding fundus area was proven by SD OCT.

**Conclusion** Coloured photopsias in dark, a black positive scotoma in bright environs are salient symptoms of this upper hemifield occult retinopathy. OCT, visual fields and electrodiagnostic findings point towards a variant of AZOOR (acute zonal occult outer retinopathy).

• T031

**The effect of vitreous opacities on stray light measurements**

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**Purpose** To evaluate in patients with unilateral complaint of floaters there is an increase in stray light measurements as compared to the contralateral eye.

**Methods** A retrospective series of patients with a unilateral complaint of floaters having undergone a straylight measurement (SM) with a C-Quant (Oculus, Germany). Data was collected on age and status of the lens. Patients having undergone vitreous surgery in either eye were excluded. The contralateral unaffected eye measurements were used as controls. A second control group consisted in patients with a posterior vitreous detachment (PVD).

**Results** Thirty-four study eyes (n=34) were included, 11 with a diagnosis of PVD and 23 with floater complaints. The mean age for all eyes (floaters and PVD) was 59.29 years, with no statistically significant differences between groups. Distributed by sex, 14 cases were males, and 20 females. Mean straylight values in the eyes with floaters was of 1.436 (SD=0.208), in the control eye 1.258 (SD=0.199); p<0.01. Straylight in eyes with a PVD 1.241 (SD= 0.251) were similar to the control eye 1.197 (SD=0.139) and did not reach statistical significance.

**Conclusion** Straylight measurements are increased in the eyes with symptomatic floaters. A PVD even when clearly visible on OCT does not increase retinal straylight.

• T032

**The prediction error of the visual field sensitivity is large at the steep 'border' of glaucomatous scotoma**

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**Purpose** To create a method to identify the edge of visual field (VF) scotoma and to investigate the relationship between the steepness of the 'border' of scotoma and the prediction error of VF sensitivity.

**Methods** Twenty-two glaucomatous patients with reliable VFs were recruited. Then, using the latest VFs (Humphrey 24-2) of these patients, the gradient of the plane on the hill of vision is calculated from the sensitivity of adjacent four/three points, so that the 'border' of VF damage is identified. Next, VF measurement was carried out, adding ten test points at the centre of adjacent four/three points where the gradient of the plane is largest (Full threshold, Custom mode). The VF measurements were performed using two approaches; random target presentation of 62 test points together and showing ten added points following or prior (randomly selected) to 52 (24-2) points. Each measurement with each approach was repeated twice in a same visit. Then, the absolute value of the difference between the measured sensitivity of the added ten test points and the average of the sensitivities of surrounding three/four test points were calculated. Finally, the relationship between the gradient of sensitivity plane and the absolute difference was investigated using the multilevel modeling (MLM).

**Results** MLM revealed significant positive relationship between the absolute difference and the gradient of plane with all of the four measurements (p<0.01).

**Conclusion** It may be advantageous to increase the spatial information by carrying out additional measurement at the gap where the border of scotoma is steep.

• T033

**Progression of visual field in patients with primary open-angle glaucoma (1): Preliminary results**

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**Purpose** To evaluate the visual field rate of progression of patients with treated ocular hypertension (OHT) and primary-open angle glaucoma (POAG).

**Methods** From a multicentric database, 441 eyes of 228 patients with treated ocular hypertension or POAG followed up at least 6 years with Humphrey 24.2 Sita-Standard visual field examination at least twice a year were identified. From initial data, eyes were classified in 5 groups: 104 ocular hypertension, 205 early glaucoma (MD>-6dB), 45 moderate glaucoma (MD -6 to -12dB), 41 advanced glaucoma (MD -12 to -18dB) and 46 severe glaucoma (MD<-18dB). Rate of progression during the follow-up period was calculated using the trend analysis of the Guided Progression Analysis software.

**Results** The mean duration of follow-up was 8.4 years. We found a significant positive association between initial MD and rate of progression in early, moderate and advanced glaucoma ( $p<0.04$ ). Rate of progression was -0.06 dB/year (-0.19 %VFI/year) in eyes with ocular hypertension, -0.24 dB/year (-0.75 %VFI/year) in eyes with early glaucoma, -0.45 dB/year (-1.72 %VFI/year) in moderate glaucoma, -0.54 dB/year (-2.44 %VFI/year) in advanced glaucoma, and -0.45 dB/year (-1.97 %VFI/year) in severe glaucoma.

**Conclusion** In early to advanced stages of glaucoma, the rate of progression worsened as the severity increased, but became smaller in latest stage of the disease.

• T034

**Secondary glaucoma in familial amyloid polyneuropathy**

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**Purpose** To describe the clinical features of secondary glaucoma associated with transthyretin (TTR)-related Familial Amyloid Polyneuropathy (FAP).

**Methods** In this retrospective monocentric study, 5 patients with FAP associated secondary glaucoma were seen at the ophthalmologic consultation of the french national center for FAP between 2011 and 2012. The mutation of the amyloidogenic TTR variants was analysed for all patients. All patients had a complete ophthalmic examination including BCVA, IOP, slit lamp and optic disc photographs, gonioscopy, pachymetry, automated perimetry, and OCT-RNFL. Glaucomatous optic neuropathy was diagnosed based on the presence of visual field abnormalities, neuroretinal rim thinning, excavation or RNFL defects. Medical and surgical treatments were analysed for all patients.

**Results** All cases had bilateral involvement except 2 monophtalmic patients. All patients were of portuguese origin and carriers of the Val30Met mutation. There were 4 women and 1 man with a mean age of  $58.8 \pm 7.7$  years. Mean BCVA was  $0.83 \pm 0.9$  LogMAR. Three eyes had a BCVA below 20/400. Mean IOP was  $26.6 \pm 6.6$  mmHg. Mean deviation was  $-17.6 \pm 11.2$  dB. Fringed pupil and anterior chamber amyloid deposition were noted in all affected eyes. Three patients had concomitant vitreous involvement. Patients were treated with  $1.2 \pm 1.5$  ocular hypotensive drugs. Four eyes had been treated with at least 1 filtrating surgery.

**Conclusion** FAP-associated secondary glaucoma is a very severe disease, associated with amyloid deposits in the anterior chamber and characteristic pupil deformation. Systematic and comprehensive eye examination should be performed in all patients affected with FAP in order to improve early detection of glaucoma.

• T035

**Pituitary macroadenoma misdiagnosed as advanced normal tension glaucoma**

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**Purpose** To present the clinical case of a patient with a giant pituitary adenoma that was misdiagnosed for several years and treated as a progressive NTG (Normal Tension Glaucoma).

**Methods** A 57 years old male had his first ocular examination in December 2008 and was diagnosed with NTG and started on topical treatment. The patient was declared unable to perform automated perimetry, so he was referred for Goldmann perimetry. He had several ocular exams over a period of four years, during which the treatment was gradually increased to maximal topical therapy, but the patient still developed a progressive impairment of the visual acuity, visual field and fundus aspect.

**Results** The patient was referred to our clinic in August 2012 for surgical management of his bilateral severe progressive glaucoma. During the hospital admittance, we performed automated perimetry that showed hemianopsia; he was referred afterwards for cerebral MRI and diagnosed with giant extensive pituitary adenoma. The patient had surgery, but he continued to lose visual acuity and optic atrophy installed at both eyes.

**Conclusion** NTG should remain an exclusion diagnosis (after excluding a chiasm compression as well).

• T036

**Effectiveness of the glaucoma screening in employees of the University Hospital St. Luc, UCL, in Brussels**

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**Purpose** To assess the effectiveness of free glaucoma screening for the employees of University Hospital St. Luc, UCL, Brussels, during the World Glaucoma Week 2013.

**Methods** During 1 week, the employees in the university hospital St.Luc, were invited to Glaucoma outpatients' free screening consultation. 320 responded positively. The participants filled before the examination a questionnaire: age, sex, personal ocular trauma or surgery, and familial history was asked. The subjects were examined by an ophthalmologist in training and supervised by an experienced glaucoma consultant if needed (SP). The examination of anterior segment (Van Herick's depth, Krukenberg's spindle, iris transillumination and pseudoexfoliation), the optic disc (size, rim and peripapillary hemorrhages) and the IOP by GAT were performed. Depending of clinical examination, a visual field test was performed. Finally, according to the results, the examiner classified the subjects in 'Normal' suspect' and 'Glaucoma' group. A second appointment was scheduled for the patients in the 'Suspect' or 'Glaucoma' classification.

**Results** From the 640 eyes, there were in total 15% in 'suspect group' classified. The aspect of their optic disc and a suspect visual field was the main reason for this classification. 5% of the total eyes undergone a preventive iridotomy, none of these patients had experienced any ocular symptoms. 3% of all eyes showed glaucoma with structural and functional glaucomatous damage.

**Conclusion** Glaucoma is still not widely known. Even for the employees of a large university hospital with easy access to the care, screening is important. The preventive iridotomy was the best result of this WGW screening.

• T037

**24-hour intraocular pressure rhythm in young healthy subjects evaluated with continuous monitoring using contact lens sensor**

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**Purpose** To evaluate 24-h intraocular pressure (IOP) rhythm reproducibility during repeated continuous 24-h IOP monitoring with non-contact tonometry (NCT) and a contact lens sensor (CLS) in healthy subjects.

**Methods** Twelve young healthy subjects were housed in a sleep laboratory and underwent four 24-h sessions of IOP measurements over a 6-month period. After randomized attribution, the IOP of the first eye was continuously monitored using the CLS Sensimed TriggerFish® and the IOP of the fellow eye was measured hourly using the Pulsair Intellipuff® non-contact tonometer. A nonlinear least-squares dual harmonic regression analysis was used to model the 24-h IOP rhythm. Comparison of acrophase, bathyphase, amplitude, the midline estimating statistic of rhythm (MESOR), IOP values, IOP changes and agreement were evaluated in the two tonometry methods.

**Results** A significant nyctohemeral IOP rhythm was found in 31 out of 36 sessions (86%) using NCT and in all sessions (100%) using CLS. Hourly awakening during NCT IOP measurements did not significantly change the mean phases of the 24-h IOP pattern evaluated using CLS in the contralateral eye. Throughout the sessions, intraclass correlation coefficients (ICCs) of the CLS acrophase (0.6 [0–0.9]; p=0.03), CLS bathyphase (0.7 [0.1–0.9]; p=0.01), NCT amplitude (0.7 [0.1–0.9]; p=0.01) and NCT MESOR (0.9 [0.9–1]; p<0.01) were significant.

**Conclusion** The CLS is an accurate and reproducible method to characterize the nyctohemeral IOP rhythm in healthy subjects but does not allow to estimate the IOP value in millimeters of mercury corresponding to the relative variation of the electrical signal measured.

• T038

**Sleep apnea syndrome screening in patients with normal-tension glaucoma**

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**Purpose** To determine the prevalence of sleep apnea syndrome (SAS) in patients with normal-tension glaucoma (NTG) and to evaluate the SAS screening in patients with NTG.

**Methods** Prospective study of 35 patients with NTG. Patients answered an abbreviated questionnaire of SAS : louder snoring, long silent period during which there is no breathing, night awakenings and excessive daytime sleepiness. Then, the patients answered the Epworth sleepiness scale. All patients were tested by the sleep clinic and received a dual recording RU-Sleeping®/ polysomnography for the diagnosis of SAS. The RU-Sleeping® is an interesting medical device easy to use, lightweight, compact and economic. It allows SAS screening at home and it permits to determine with the Epworth scale, patients first go into sleep recording.

**Results** The mean age of patients was 73 years +/-1.4 years. The positive predictive value (PPV) of abbreviated questionnaire was 54%. And the PPV of Epworth scale was 50% (33% of them had a Epworth score of 8-14 and 17% had a Epworth score of 15 and above). The apnea/hypopnea index (AHI) was 36.9/h. The mean oxygen saturation was 85.5%. The PPV of RU-Sleeping for SAS screening was 78%. The SAS prevalence in patients with NTG was 60%, and 66 % of them were severe (AHI>30).

**Conclusion** Routine screening SAS should be recommended to any patient with normal-tension glaucoma. So, in case of NGT diagnosis in ophthalmology, a simple interrogation evocative associated with a RU-Sleeping® could determine the patients to take charge of a priority especially in case of shortage of beds recordings. Our preliminary results suggest high prevalences of NTG or SAS, a larger sample in this prospective study will allow to confirm it in the future.

• T039

**Comparing the use of steroid vs artificial tears following selective laser trabeculoplasty**

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**Purpose** To compare between using potent steroid, mild steroid or just artificial tears following selective laser trabeculoplasty (SLT), and its effect on outcome

**Methods** Retrospective file review of all patients underwent SLT in our institute for a year; the data divided into 3 groups: group 1 received Prednisolone acetate 1% ophthalmic suspension ± their glaucoma medications, in the immediate post laser period, group 2 had Fluorometholone eye drops, and group 3 had artificial tears. The rate of complication & outcome was compared between the groups.

**Results** 215 eyes of 129 patients underwent SLT, from October 2012-October 2013. During the post-laser period, Prednisolone acetate 1% was used in 134 eyes, for 8.79±3.6 days (3-14 days), Fluorometholone for 41 eyes for 6.85±2.1 (3-14 days) & 40 patients had neither. Most of the eyes had 360° treatment, half of the patients had DM & 40% had hypertensionPre-SLT intraocular pressure(IOP) in group 1 was 19±6mmHg on 2.4 glaucoma medications, in group 2 19.4±5.4mmHg on 2.4 medications & in group 3 was 21±6.2mmHg on 2.5 medications.The first group treated with 78.6±13.2 shots of 0.56mj per shot, group 2 with 81.1±16.6 shots of 0.59mj, and group 3 with 75.9±15.2 of 0.59mj.The resulted decrease of IOP in the first group was 2.4±5.3mmHg (12.7%), in group 2 was 3.4±4.8mmHg (17.7%) & in the last group was 5.5±6.1mmHg (26.1%).One patient had persistent anterior chamber reaction for more than 2 week, in both eyes, in the first group( that was treated with prednisolone). In the other groups, no eye showed any degree or AC reaction.

**Conclusion** The use of steroid had an adverse effect on the result of SLT, while the use of artificial tears post-SLT did not result in increased rate of complication.

• T040

**Comparative effectiveness of bimatoprost 0.03% preservative free for the treatment of open-angle glaucoma and ocular hypertension**

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**Purpose** To evaluate the comparative effectiveness of bimatoprost 0.03% preservative-free solution (BimPF) in single dose vials for the treatment of glaucoma/ocular hypertension compared to other preservative-free (PF) and non-BAK preserved prostaglandin analog and prostamide (PGA) therapies. Primary outcome of interest was change from baseline in intraocular pressure.

**Methods** A systematic literature review was conducted to identify relevant randomized controlled trials investigating the IOP lowering efficacy of glaucoma treatments. The initial literature search was done in December 2011, and subsequently updated in April 2013. Trials involving preserved formulations were included to indirectly connect the efficacy evidence of PF and non-BAK preserved PGA therapies. A Bayesian mixed treatment comparison was used to synthesize the network of evidence. Timolol was used as the reference comparator, as it was the most common comparator in the glaucoma medication trials.

**Results** The initial literature search resulted in 151 trials to be included in the network. The updated literature search resulted in the addition of 4 additional trials. In total, 84 comparator arms were included in the network. Relative to timolol, BimPF showed a significant difference in IOP lowering efficacy relative to timolol, while all other PF and non-BAK preserved PGA therapies did not. Additionally, BimPF showed the greatest mean change from baseline in IOP compared to all the PF and non-BAK preserved PGA therapies, although the differences were non-significant.

**Conclusion** BimPF is the only PF or non-BAK preserved PGA therapy to show a significantly greater IOP lowering efficacy than timolol.

• T041

**Comparative effectiveness of bimatoprost 0.03% / timolol 0.5% preservative free fixed combination (BTFC PF) for the treatment of open-angle glaucoma and ocular hypertension**

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**Purpose** To evaluate the comparative effectiveness of BTFC PF solution in single dose vials for the treatment of glaucoma/ocular hypertension compared to other preservative-free (PF) fixed combination and PF monotherapies. Primary outcome of interest was change from baseline in intraocular pressure.

**Methods** A systematic literature review was conducted to identify relevant randomized controlled trials investigating the IOP lowering efficacy of glaucoma treatments. The literature search was performed in December 2012. Trials involving preserved formulations were included to indirectly connect the efficacy evidence of PF treatments. A Bayesian mixed treatment comparison was used to synthesize the network of evidence.

**Results** The initial literature search resulted in 148 papers meeting the inclusion criteria. Of these 135 could be connected to form the evidence network, which consisted of 23 comparator arms, 6 of which were PF fixed combinations or PF monotherapies. BTFC PF showed an improvement in IOP lowering efficacy relative to all other PF therapies and the greatest mean change from baseline in IOP, although this difference was non-significant for 3 out of 5 comparisons, likely due to limited evidence for comparator PF therapies. The analysis showed a 93% probability that BTFC PF was the best of the PF treatments based on the full network analysis. All other PF therapies have a <3% chance of being best.

**Conclusion** The MTC analysis showed numerical superiority of BTFC PF over all other PF treatments within the network of evidence for combination therapies.

• T042

**Comparative analysis of hyperaemia rate between preservative-free latanoprost and preserved prostaglandin eyedrops. An adjusted indirect comparison meta-analysis**

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**Purpose** For the evaluation of hyperaemia, differences in measurement process could exist between the trial in terms of definition, procedure, follow-up, etc. These differences make the comparison of absolute rate or proportion of patients with hyperaemia between the trial inappropriate and potentially confused by these differences. To avoid this problem, meta-analysis does not pool absolute proportion but odds ratio (or risk ratio). Odds ratios make implicit adjustment for this kind of difference by using the value observed in the control group as reference. The technique of "adjusted indirect comparisons" are based on this principle and were used to assess tolerability of a preservative free latanoprost compared to preserved prostaglandins for the treatment of open-angle glaucoma (OAG) and ocular hypertension (OH).

**Methods** The meta-analysis was performed according to a protocol established before the start of the literature search and data analysis. It was conducted and reported according to the recent PRISMA statement. The main endpoints were intraocular pressure (IOP) and hyperaemia

**Results** Twenty-one studies were included. The risk of hyperaemia was statistically significantly lower with preservative-free latanoprost than with polyquaternium-1-travoprost (OR [95%CI]: 0.24 , sofzia-travoprost (0.37 ), BAK-bimatoprost 0.03% (0.18 ), BAK-bimatoprost 0.01% (0.27 ), BAK-tafluprost (0.18 ), BAK-travoprost (0.25 ) and BAK-latanoprost (0.52 ]

**Conclusion** The risk of hyperaemia was found statistically significantly lower with preservative free latanoprost than with all the preserved prostaglandin analogs comprised in the metaanalysis.

*Commercial interest*

• T043 / 4226

**Removal of preservative from Ganfort improves intraocular pressure (IOP) lowering in patients – A timolol dose-response phenomenon**

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**Purpose** A benzalkonium chloride (BAK)-free fixed-combination formulation of bimatoprost and timolol (Ganfort) has been developed for patients who are sensitive to preservatives. A randomized controlled study in 561 patients compared the IOP-lowering efficacy of the BAK-free formulation versus the preserved Ganfort. At all timepoints, IOP lowering, numerically, consistently favoured the BAK-free formulation. This finding contradicted expectations that the removal of BAK would result in lower ocular bioavailability of both bimatoprost and timolol, resulting in less efficacy. Research was conducted to explore possible explanation for the clinical observation.

**Methods** Literature search on timolol dose response was performed, focusing on IOP lowering following topical dosing of 0.1%, 0.25%, and 0.5% timolol ophthalmic solutions.

**Results** A U-shaped dose-response curve exists for timolol, and available data suggest that the dose that elicits maximal IOP lowering lies between 0.25% and 0.5%.

**Conclusion** Removal of BAK from the fixed combination product Ganfort, which contains 0.5% timolol, may have resulted in a lower, but optimal, ocular concentration of timolol, leading to improved IOP-lowering efficacy with the preservative-free formulation.

*Commercial interest*

• T044 / 4227

**SYL040012, a siRNA for the treatment of glaucoma**

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**Purpose** SYL040012 is a siRNA under developed for the treatment of glaucoma and increased intraocular pressure (IOP). siRNAs are small double stranded RNAs that regulate protein expression at transcription level using the endogenous RNA interference machinery. Therapeutically siRNAs are attractive due to their specificity, potency and ability to silence targets that are not addressable by small molecules. SYL040012 targets ADRB2, a well-known target for glaucoma.

**Methods** Silencing activity of SYL040012 was validated in cell lines and IOP lowering efficacy was studied in rabbit. Silencing activity was assessed by extracting total RNA from cells or animal tissues and analyzed by real time-PCR. IOP was measured using applanation tonometry. Tissue and plasma exposure was evaluated following a single instillation to rabbits using a non-denaturing Anion Exchange HPLC combined with fluorescence detection. 28-day regulatory toxicology studies were performed in cynomolgus monkeys following a daily ocular instillation of one of three doses of SYL040012 or vehicle. Phase I clinical trials were performed in healthy volunteers or individuals with increased IOP that received either a single dose or seven repeated administrations of SYL040012.

**Results** SYL040012 reaches structures of the eye relevant to the treatment of glaucoma when administered in eye drops; but is only detected in systemic circulation and non-ocular tissues at trace levels. Presence of this compound in the ciliary body it results in specific down-regulation of the target gene and reduction in IOP in animal models. Preclinical and clinical studies show that SYL040012 is well tolerated both systemically and locally.

**Conclusion** In summary, SYL040012 is a promising candidate for the treatment of increased intraocular pressure associated to glaucoma.

*Commercial interest*

• T045 / 4225

**Patient behavior when prescribed non-affordable glaucoma medication in the medical unit, National Research Center, Cairo, Egypt**

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**Purpose** (1)Identify cost-reducing strategies,including cost related non-adherence(CRN),employed by primary open angle glaucoma (POAG)patients;(2) explore impact of doctor-patient interaction(DPI)on CRN and(3)pinpoint best DPI methods that minimized CRN.

**Methods** A Cross-sectional study was enrolled on consecutive adult patients attending the eye clinic of the National Research Center,Cairo,Egypt, diagnosed with POAG and on outpatient topical anti-glaucoma drugs in the first 8 months of 2012.Data were collected via an interviewer-administered questionnaire.The impact of factors as education,insurance and drug cost to income ratio were tested statistically.

**Results** Data gathered from 280 patients showed that 75% of patients employed at least 1 method of CRN(termed CRN+).Of those, up to 70% skip doses,64% postpone buying prescriptions and 28% ignore buying it altogether.We found significant relationships between lower CRN scores and better insurance coverage( $p=0.002$ ),higher education( $p=0.002$ ),lower values of "drug cost to monthly income" ratio( $p=0.003$ ),lower number of prescriptions( $p=0.003$ )and better DPI regarding drug costs( $p=0.004$ ). 58% of CRN+ patients reported having DPI,(termed CRN+DPI).In CRN+DPI, the most common methods were doctors showing sympathy towards patients' drug costs(72%),ensuring patients' affordability of drugs(65%)and change one to a cheaper alternative(62%).In those who did not have DPI,the most cited cause was "being embarrassed" to ask about drug costs(80%).

**Conclusion** Future policies should focus on improving DPI to lower CRN,mainly,ensuring patient education on drug costs, showing strong emotional support and reducing polypharmacy. Encouraging patients to ask for cheaper alternatives can also help.

• T046

**Outcome of fornix-based versus limbal-based conjunctival flaps in trabeculectomy**

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**Purpose** To compare the surgical outcomes and bleb morphology of limbal- based group with that of fornix-based group undergone trabeculectomy with mitomycin C (MMC).

**Methods** 50 eyes of 50 patients with trabeculectomy with MMC who were observed for 1 year were included in the study. A limbal-based conjunctival flap was used for 25 eyes of 25 patients and a fornix-based conjunctival flap for the other 25 eyes of 25 patients. We classified and compared the bleb morphology according to the Moorfield Bleb Grading Sistem after 1 year postoperatively, and evaluated intraocular pressure, success rates.

**Results** There was no significant difference in the IOP and success rate in two groups. The central bleb vascularity of the limbal-based group was statistically lower than that of fornix-based group ( $1.69 \pm 0.56$  :  $2.08 \pm 0.69$ ,  $p=0.042$ ). The risk of cystic bleb formation was higher in the limbal-based group ( $34.2\%$  :  $14.6\%$  ,  $p = 0.047$ ).

**Conclusion** There was no difference between the groups in the IOP and cumulative success rate, but fornix-based group was recommendable concerning the low risk of cystic bleb formation.

• T047

**Ultrasonic circular cyclo coagulation in patients with primary open-angle glaucoma: A multicenter clinical trial**

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**Purpose** To evaluate the efficacy and safety of the Ultrasonic Circular Cyclo Coagulation (UC3) procedure in patients with primary open-angle glaucoma (POAG).

**Methods** Prospective multicenter clinical trial. 42 eyes of 42 patients with POAG, intraocular pressure (IOP) > 21 mmHg, an average of 1.65 failed previous surgeries and an average of 3.2 hypotensive medications were insonified with a probe comprising 6 piezoelectric transducers. 18 patients (group 1) were treated with a 4 seconds exposure time for each shot and 24 patients (group 2) with a 6 seconds exposure time. Follow-up visits were performed at 1 day, 1 week, 1, 2, 3, 6 and 12 months after.

**Results** IOP was significantly reduced in both groups ( $p<0.05$ ), from a mean preoperative value of  $28.6 \pm 4.7$  mmHg in group 1 and  $28.1 \pm 8.6$  mmHg in group 2 to a mean value of  $16.1 \pm 2.8$  mmHg in group 1 and  $16.7 \pm 4.4$  mmHg in group 2 at last follow-up. Success (IOP reduction >20%) was achieved in 12 of 18 (67%) eyes of the group 1 and in 17 of 24 (71%) eyes of the group 2. Four patients were re-treated. No major intra- or post-operative complications occurred.

**Conclusion** UC3 seems to be an effective and well-tolerated method to reduce intraocular pressure in patients with POAG.

**Commercial interest**

• T048

**Comparison of Esnoper® implant placement in non penetrating deep sclerectomy**

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**Purpose** To compare the efficacy and safety of the Esnoper® implant in non penetrating deep sclerectomy (NPDS) with the implant placed in two different positions: under the scleral flap (in the scleral lake) versus a portion of the implant introduced in the suprachoroidal space.

**Methods** Retrospective analysis of 40 eyes of 30 patients who underwent NPDS, being the implant Esnoper® placed under the scleral flap in Group A (22 eyes) and a part of it in suprachoroidal space in Group B (18 eyes).

**Results** In group A, the IOP decreased from  $22.8 \pm 4.2$  mmHg to  $15.1 \pm 2.9$  mmHg and a group B from  $23.4 \pm 3.1$  mmHg to  $13.9 \pm 3.1$  mmHg ( $p < 0.05$ ). The difference in the postoperative IOP between both groups was statistically non significant ( $p > 0.05$ ). Anti-glaucoma medications decreased from  $2.81 \pm 0.5$  to  $0.8 \pm 0.1$  in group A, and from  $2.9 \pm 0.5$  to  $0.2 \pm 0.4$  in group B, being this difference statistically significant ( $p < 0.05$ ). Qualified success was found in all patients (100%) in both groups. Complete success (IOP without treatment) was achieved in 14/22 (63.3%) eyes in group A and in 15/18 (83.3%) eyes in group B. The average follow up was 1 year.

**Conclusion** The IOP-lowering effect and safety of scleral and suprachoroidal Esnoper® implant seem to be comparable, but fewer anti-glaucoma medications were required with the implant partially introduced in the suprachoroidal space.

• T049

**Treatment of refractory glaucoma using UC3 procedure with HIFU (High Intensity Focused Ultrasound). Prospective series**

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**Purpose** To assess the safety and efficacy of Ultrasound Circular Cyclo-Coagulation (UC3 procedure) using HIFU (high intensity focused ultrasound) in patients with refractory glaucoma.

**Methods** Prospective clinical series performed in two centers, on twenty-seven eyes of twenty-seven patients with refractory glaucoma, treated with the EyeOP1 medical device equipped with six miniaturized cylindrical piezoelectric transducers. All eyes were treated with a 6-second exposure time from each transducer. The main assessment criteria were safety and efficacy as indicated by the incidence of complications and by the IOP reduction. Ophthalmic, ultrasound biomicroscopy and flare examinations were performed before treatment and during clinical follow-up at D1, D7, M1, M2, M3 and M6

**Results** No major intra- or post-operative complications occurred. Clinical examination showed no lesions of the ocular structures other than the ciliary body and no or few signs of intraocular inflammation after treatment. Visual acuity was not modified after the procedure. The mean intraocular pressure was reduced from  $27.5 \pm 7.0$  mmHg before treatment to  $18.3 \pm 4.4$  mmHg at last follow-up. Four patients needed to be re-treated. The success rate, as defined by an IOP reduction >20%, was 67%. The mean IOP reduction achieved was 33%. Ocular inflammation evidenced by flare was very limited and non-significant.

**Conclusion** Coagulation of the ciliary body using high intensity focused ultrasound delivered by miniaturized transducers is a simple, well-tolerated procedure which enables to significantly reduce the intraocular pressure in patients with refractory glaucoma.

*Commercial interest*

• T050 / 3726

**Argon laser trabeculoplasty (ALT) for advanced glaucoma: A 12 months follow-up study**

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**Purpose** ALT has been used for lowering the intraocular pressure (IOP) for over 25 years. The aim of this study was to investigate the safety and efficacy of ALT in cases of advanced glaucoma unsuitable for surgery due to ocular or systemic conditions.

**Methods** This retrospective observational study had the following inclusion criteria: advanced POAG with uncontrolled IOP, maximum medical treatment, previously failed ALT or filtration surgery and unsuitability for surgical intervention due to ocular or systemic comorbidities. The data collected included: pre- and postoperative number of medications, visual acuity (VA), visual fields mean deviation (VF MD), IOP and adverse events during the study period. All ALT treatments were carried out by the same clinician.

**Results** A total of 40 patients (40 eyes; mean age: 79 years; range 56-92 years) were identified. The number of medications remained unchanged in 75% patients, decreased by 2-4 medications in 12.5% of patients and increased by 1-2 in 12.5% of patients. Mean Log MAR VA (baseline  $0.27 \pm SD 0.45$ ; final  $0.19 \pm SD 0.3$ ) and the VF MD (baseline  $-9.17 \pm SD 8.9$ ; final  $-9.45 \pm SD 9.3$ ) did not change significantly over the 12 months study period (Log MAR p=0.29; VF MD p=0.18). The change in IOP was statistically significant,  $p=0.00018$  ( $p<0.001$ ), with an average IOP reduction by 18.14% at 12 months. In 76% of patients the mean IOP decreased by 6 mmHg and in 47.5% of patients the final IOP was lower than 16mmHg. No adverse events were observed during the study.

**Conclusion** ALT appeared safe and effective in lowering the IOP in cases of advanced glaucoma unsuitable for surgery.

• T051

**Neovascular glaucomas after central retinal artery occlusions**

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**Purpose** Neovascular glaucoma after acute central retinal artery occlusion (CRAO) is underestimated. It is a serious complication, that can be prevented.

**Methods** This retrospective study included all consecutive cases treated in Caen University Hospital (France), between January 2010 and December 2012. We studied the incidence of neovascular glaucoma in this cohort, the delay of onset, and the degree of arterial repermeabilisation on fluorescein angiography. Patients with pre-existing ocular neovascularisation or evidence of ocular ischemic syndrome were excluded. We reviewed the literature about the diagnostic, therapeutic and epidemiological aspects of neovascular glaucoma after CRAO.

**Results** Among thirty one patients with acute central retinal artery occlusion, five developed neovascular glaucoma (16%). These five patients had poor revascularisation, that was not diagnosed by the fundus examination. The time interval between the occlusive event and the neovascular glaucoma ranges from 7 weeks to 7 months. Patients were all treated with topical antiglaucoma drops. A panretinal photocoagulation (PRP) was done in 4 cases, associated with intravitreal bevacizumab injection in 2 cases, and with a transscleral cyclophotocoagulation with diode laser in one case.

**Conclusion** Neovascular glaucoma is the height of CRAO. To prevent it, a fluorescein angiography should be performed a few days after each CRAO to evaluate the repermeabilisation. If it is not good, a PRP should be quickly started, associated with a close follow up.

• T052

**Imaging of the optic nerve (oct rnfl and ganglion cells analysis) in monitoring the child's congenital glaucoma**

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**Purpose** Apply monitoring child's congenital glaucoma, imaging of the optic nerve of adult glaucoma tests: RNFL and analysis of ganglion cells in Optical coherence tomography.

**Methods** We studied 30 children with congenital glaucoma balanced aged 6 to 18 years whose usual monitoring focuses on visual acuity, intraocular pressure and visual field. Using Zeiss Cirrus HD OCT Spectral Domain, we studied the structure of the optic nerve of these patients (RNFL and ganglion complex) and was compared to that of adults.

**Results** The values obtained for young patients with normal visual field are identical to those of an adult an average of  $99 \mu m$  for the RNFL and  $85 \mu m$  for ganglion cells analysis. For other patients anomalies are consistent with campimétrie deficits observed. Imaging of the optic nerve found the same abnormalities of function and structure of the optic nerve in adults. Thanks to the rapid acquisition of OCT images, this review is implementation easier and earlier than the visual field study in children. OCT allows monitoring of glaucoma patients for several years. However, in children, this monitoring is not carried out in practice common and could serve as an additional tool.

**Conclusion** Child's congenital glaucoma requires regular monitoring based on intraocular pressure and when age permits the study of the visual field. The monitoring of these patients can be started earlier with the study of the optic nerve in Oct (RNFL and ganglion cells analysis) to the consistency of results between adults and children. The completion of the visual field is difficult before the age of 8, the feasible before OCT this age allows earlier monitoring of this disease.

• T053

**Hemifield pattern electroretinogram, frequency doubling technology and optical coherence tomography for detection of early glaucomatous optic neuropathy in ocular hypertensive patients**

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**Purpose** To assess which is the most sensitive and specific exam for detecting early glaucomatous damage in ocular hypertensive patients (OH) among hemifield test pattern electroretinogram (PERG HF), frequency doubling technology (FDT) and optical coherence tomography retinal nerve fiber layer (OCT RNFL).

**Methods** Fifty-two OH patients (mean age of  $56 \pm 9.6$  yrs) with an intraocular pressure (IOP)  $> 21$  mmHg and 52 healthy controls (mean age of  $54.8 \pm 10.4$  yrs) with IOP  $< 21$  mmHg were assessed. All the patients had normal visual acuity, normal optic disc appearance and normal standard achromatic perimetric (SAP) indices. All subjects underwent OCT, FDT and PERG examination. Data were analyzed with Mann-Whitney test and Receiver Operating Characteristic (ROC) curve analysis.

**Results** OH patients showed thinner OCT-RNFL than controls, especially in the superior and inferior quadrant (respectively,  $130.16 \pm 10.02$  vs  $135.18 \pm 9.27$   $\mu\text{m}$ ;  $p < 0.011$  and  $120.14 \pm 11.0$  vs  $132.68 \pm 8.03$   $\mu\text{m}$ ;  $p = 0.001$ ) and a significantly higher FDT pattern standard deviation (PSD) ( $3.46 \pm 1.48$  vs  $1.89 \pm 0.7$  dB;  $p = 0.001$ ). As regards PERG examination, the amplitude of the N95 wave of the lower and upper PERG HF showed significant differences (respectively,  $p = 0.037$  and  $p = 0.023$ ) between the two groups. ROC curve analysis revealed a sensitivity of 92% and specificity of 86% for FDT PSD (with an area under the ROC curve of 0.942), whereas by using OCT

**Conclusion** Our study show that FDT and PERG HF are the most sensitive and specific exams for detecting early glaucomatous damages in eyes with OH and can be useful in identifying those patients who may develop glaucoma and who need hypotensive ocular medications.

• T055

**Structure function relationship: Specific index of ganglion cell complex assessment of 2 spectral domain OCT and standard automated perimetry**

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**Purpose** To investigate structure function relationship between specific parameters of structural damage analyzed by OCT RTVue100 (Optovue, Inc.) and CirrusHD-OCT (Carl Zeiss Meditec) with functional parameters of standard automated perimetry (SAP).

**Methods** 143 open angle glaucoma and 43 control eyes were included. They underwent a macular grid exam with RTVue100, CirrusHD-OCT and 24°2 and 10°2 SAP (Humphrey SITA-standard). Specific structural parameters analyzed were Global and Focal Loss Volume (GLV and FLV) with RTVue, average and minimum Ganglion Cell and Inner Plexiform Layer (GCIPL) thickness with CirrusHD-OCT. Functional parameters collected were MD, PSD and VFI for 24°2 SAP and MD and PSD for 10°2 SAP. A Student t-test was performed to compare parameters and a logistic regression to calculate Pearson coefficient ( $r$ ) studying correlation between structural and functional parameters.

**Results** Glaucoma group had a significant functional (in 24°2 and 10°2 SAP) and structural impairment compared to control. Logistic regression analysis found a linear correlation between structural and functional parameters. The highest Pearson coefficient with OCT RTVue was between FLV and PSD in 24°2 SAP ( $r = 0.72$ ) and for CirrusHD-OCT between minimum GCIPL and PSD in 24°2 SAP ( $r = 0.56$ ).

**Conclusion** This study objectives a structure function relationship between specific focal structural indices (FLV and GCIPL-Min) assessed with these 2 SD-OCT and functional (PSD) parameters damage. It would be interesting to confirm this new approach of SFR with these specific structural parameters.

• T054

**Backwards compatibility of HRT3 and HRT II before and after cataract surgery and intraocular lens implantation**

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**Purpose** To assess the backwards compatibility of HRT3 and HRT II examinations in cataractous eyes before and after surgery.

**Methods** 43 patients waiting for first eye cataract surgery, 12 men and 31 women aged  $75 \pm 5$  years, took part in the study. Optic nerve head (ONH) topographies were performed before cataract surgery and one month postoperatively using the HRT II software. These examinations were later accessed using the HRT3 software. The calculation of topography, the topography standard deviation (TSD), and the image alignment of the preoperative and the postoperative scans were evaluated. TSD  $\leq 50$  was considered acceptable.

**Results** The recorded data was accessed using the HRT3 software, and in 34 cases the topography could be calculated. The mean TSD of the 34 examinations obtained of the eyes before cataract surgery was  $33 \pm 18$ . Considering TSD  $\leq 50$  as acceptable, there were 28 reliable scans. Of the data obtained one month after cataract surgery, topographies using HRT3 could be calculated in 39 of 43 examinations. TSD was  $21 \pm 12$  indicating improvement of the quality of the HRT after cataract surgery. The HRT3 software was able to calculate both the preoperative and the postoperative topography in 31 of 43 eyes (72%); three of the baseline examinations were considered unreliable because of high TSD values, though. The contour line was correctly aligned in all 31, and the image size was unchanged in all cases.

**Conclusion** Successful topographies using HRT3 software could be calculated in two thirds of the eyes both before and one month after cataract surgery with stable image alignment and image size in all cases. Follow-up of the ONH topography over cataract surgery was possible in all eyes with reliable examinations.

• T056

**Ganglion cell complex measurement and glaucoma diagnosis using two SD-OCT**

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**Purpose** Macular retinal ganglion cell analysis from Cirrus HD-OCT (Carl Zeiss Meditec, Dublin, CA) and RTVue-OCT (Optovue Inc., Fremont, USA) were evaluated in their glaucoma diagnostic ability.

**Methods** A total of 169 eyes were enrolled in 3 groups: (1) 70 early primary open angle glaucoma patients (POAG), (2) 44 moderate-to-advanced POAG, (3) 55 normal subjects. All patients underwent a complete examination and a macular analysis using GCA algorithm from Cirrus HD and GCC algorithm from RTVue. Macular average, minimum and 6 sectoral GCL-IPL thicknesses obtained with Cirrus and average, inferior and superior GCC thicknesses and FLV and GLV obtained with RTVue were analyzed. Each eye underwent cpRNFL analysis using Cirrus and RTVue. Glaucoma discrimination ability was assessed using area under the receiver operator characteristic curve (AUC) for all parameters.

**Results** Average GCL-IPL thickness with Cirrus was  $65.6 \pm 7.4$   $\mu\text{m}$ ,  $57.8 \pm 13.1$   $\mu\text{m}$  and  $80.3 \pm 9.9$   $\mu\text{m}$  and average GCC with RTVue was  $75.7 \pm 10.7$   $\mu\text{m}$ ,  $72.6 \pm 9.7$   $\mu\text{m}$  et  $93.9 \pm 7.7$   $\mu\text{m}$  for group 1, 2 and 3 respectively ( $p < 0.05$  between each group). AUCs ranged from 0.836 to 0.925 for Cirrus macular parameters and from 0.878 to 0.949 for RTVue macular parameters. These values were compared to the discrimination ability of cpRNFL parameters from each OCT.

**Conclusion** The two OCT macular ganglion cell analysis showed similar glaucoma diagnostic ability and were comparable with that of cpRNFL. Specific macular parameters: GCL-IPL minimum, GCL-IPL inferotemporal, FLV and GLV have a high diagnosis performance.

• T057

**Analyse of segmented macular inner layers by spectral domain optical coherence tomography in glaucoma**

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**Purpose** To evaluate the glaucoma discriminating ability of macular inner layers measured by SD-OCT (Cirrus HD-OCT, Carl Zeiss Meditec (CZM) Dublin, CA, USA).

**Methods** Our study concerns 252 primary open angle glaucoma (POAG) eyes (early, moderate and advanced glaucoma) and 337 normal eyes which underwent a visual field testing (Humphrey Field Analyser, SITA-Standard 24-2, CZM), and Cirrus HD-OCT imaging (macular and optic disc cube 200x200). OCT macular scans were segmented into macular nerve fibre layer (mNFL), ganglion cell layer with inner plexiform layer (GCIPL) and ganglion cell complex (GCC=GCIPL+mNFL). Area under the receiver operator characteristic curve (AUC) for macular parameters and mean circumpapillary retinal nerve fibre layer (cpRNFL) were used.

**Results** There is a statistically significant difference between glaucomatous and control eyes for all parameters ( $p < 0.05$ ). For early POAG group the best AUCs were respectively minimum GCIPL (0.747), average GCIPL (0.716), GCC (0.71), cpRNFL (0.704), minimum mNFL (0.673) and average mNFL (0.655). For moderate POAG group the best AUCs were respectively minimum GCIPL (0.864), GCC (0.858), cpRNFL (0.853), average GCIPL (0.838), average mNFL (0.82) and minimum mNFL (0.815). For advanced POAG group the best AUCs were respectively minimum GCIPL (0.938), GCC (0.928), minimum mNFL (0.92), average GCIPL (0.908), cpRNFL (0.854) and average mNFL (0.854).

**Conclusion** Minimum GCIPL would have the highest sensitivity to diagnosis glaucoma. It may represent an interesting complementary parameter to cpRNFL thickness.

• T058

**Comparaison of two anterior segment OCT: CASIA (Tomey) versus OCT Visante (Zeiss)**

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**Purpose** To compare the anterior chamber (AC) and AC angle measurements obtained with a spectral-domain and a time-domain anterior segment optical coherence tomography (AS-OCT), and to evaluate their repeatability

**Methods** Prospective cross-sectional study conducted on healthy subjects. The AC was imaged with a spectral-domain (Casia SS-100, Tomey) and a time-domain AS-OCT (Visante, Zeiss) in one eye of each patient in a random order. The central corneal thickness (CCT), anterior chamber depth (ACD), angle opening distance at 500 and 700 µm (AOD 500 and AOD 750), trabecular iris space area at 500 and 700 µm (TISA 500 and TISA 750) and scleral spur angle (SSA) were measured. The repeatability measurements were evaluated by the intraclass correlation coefficients (ICCs)

**Results** 101 eyes of healthy subjects were analyzed. The repeatability was excellent with the Casia and the Visante for CCT (ICC 0.90-0.94 and 0.95-0.97), ACD (ICC 0.95-0.98 and 0.93-0.96), AOD 500 (ICC 0.92-0.96 and 0.90-0.94) and 750 (ICC 0.93-0.96 and 0.91-0.94), TISA 750 (ICC 0.81-0.97 and 0.9-0.94) and SSA (ICC 0.9-0.94 and 0.89-0.94), and moderate for TISA 500 (ICC 0.68-0.97 and 0.7-0.93). For all parameters, Casia and Visante measurements were significantly correlated ( $r = 0.76-0.98$ ;  $p < 0.002$ ). Differences were non-significant for CCT, AOD 500 and 750, TISA 500 and 750. ACD measured with Casia was significantly larger than that measured with the Visante, and SSA measured with Casia was significantly lower than that measured with Visante

**Conclusion** Both Casia and Visante AS-OCTs demonstrate high repeatability. Excepted for the ACD and SSA, the two AS-OCTs agree well in AC and AC angle measurements

• T059

**Inter-test variability in normals and glaucomatous patients tested by Optopol PTS-910, Glaucoma program**

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**Purpose** To quantify the inter-test variability (dB) for the Optopol automated perimeter, Glaucoma Fast Threshold program, in normals and glaucoma patients.

**Methods** A prospective study was performed on 204 glaucoma patients and a control group of 30 normal subjects, tested by complete ophthalmological exam and automated perimetry (Optopol PTS-910). The visual field was tested weekly for 4 consecutive weeks, using the Glaucoma Fast Threshold strategy. The visual field defects were classified according to the Aulhorn-Karmeyer descriptive scale.

**Results** For the control group, the average inter-test variability was of  $1.57 \pm 0.24$  dB, lower next to fixation and increasing towards the 50 degree isopter. The average inter-test variability increases along with the perimetric stage:  $1.57 \pm 0.66$  dB for pre-perimetric glaucoma,  $2.13 \pm 1.04$  dB for non-specific defects group,  $3.23 \pm 1.01$  dB for the stage 1,  $3.52 \pm 2.61$  dB, for the stage 2,  $3.65 \pm 1.19$  dB for the stage 3 and  $5.82 \pm 1.67$  dB for the stage 4. For the cases of preperimetric glaucoma and non-specific defects, a similar profile of variability as for the normal subjects could be observed. For the stages 2-4, the profile of the areas with maxim inter-test variability moves towards the relative scotoma and the surrounding area.

**Conclusion** A better description of the inter-test variability and the evolution of this intricate parameter of the retinal light sensitivity is useful for the differential diagnostic between the real change and the « background noise » in early detection of the functional progression in glaucoma.

• T060

**Choroidal thickness in open-angle glaucoma**

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**Purpose** To examine choroidal thickness in open-angle glaucoma in a hospital-based case series study

**Methods** Choroidal thickness was measured by enhanced depth imaging of spectral domain optical coherence tomography. The study group included patients with open-angle glaucoma and the control group consisted of subjects with a normal fundus.

**Results** The study group included 71 eyes (39 patients) and the control group consisted of 189 patients (228 eyes) with significant difference between both groups in age ( $P=0.16$ ) and refractive error ( $P=0.07$ ). Choroidal thickness in the foveal region ( $P=0.18$ ), at a distance of 1000 µm from the fovea ( $P=0.39$ ), at a distance of 2000 µm from the fovea ( $P=0.46$ ), and at a horizontal distance of 2500 µm from the fovea ( $P=0.53$ ) did not vary significantly between both groups. In multivariable analysis with adjustment for age and refractive error, subfoveal choroidal thickness ( $P=0.12$ ; regression coefficient B: -8.60; 95%CI: -19.3, 2.1), choroidal thickness at a horizontal distance of 1000 µm from the fovea ( $P=0.30$ ; B: -4.98 (95%CI: -14.3, 4.4)), 2000 µm from the fovea ( $P=0.20$ ; B: -20.9 (95%CI: -53.2, 11.3)) and 2500 µm from the fovea ( $P=0.45$ ; B: -2.70 (95%CI: -9.67, 4.27)) was not significantly associated with the diagnosis of glaucoma. In binary regression analysis with adjustment for age and refractive error, presence of glaucoma was significantly associated neither with subfoveal choroidal thickness ( $P=0.12$ ; OR: 0.997 (95%CI: 0.993, 1.001)), nor with choroidal thickness at a horizontal distance of 1000 µm from the fovea ( $P=0.47$ ; OR: 0.998 (95%CI: 0.993, 1.002)).

**Conclusion** After adjusting for age and refractive error, open-angle glaucoma was not significantly associated with a marked thinning or a thickening of the choroid in the foveal and parafoveal region.

• T061 / 2726

**Cognitive function associated with larger optic nerve heads**

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**Purpose** To assess associations between optic nerve head size and parameters of cognitive function in a population-based study design.

**Methods** The Beijing Eye Study is a population-based cohort study in Northern China and included 4442 subjects. The study participants underwent a detailed ophthalmologic examination and an interview with questions on the level of education. Using fundus photographs, we measured the size of the optic disc.

**Results** Assessable optic disc photographs were available for 4089 (92.1%) subjects. After adjusting for age, gender, refractive error, and best corrected visual acuity, optic disc size was significantly associated with a higher level of education ( $P<0.001$ ) and shorter time needed to perform frequency doubling threshold perimetry ( $P<0.001$ ). In a reverse manner, the level of education was significantly associated with increasing optic disc size ( $P<0.001$ ) and shorter perimetric test duration ( $P<0.001$ ) after adjustment for age, gender and best corrected visual acuity.

**Conclusion** In a population-based study on adult Chinese, optic nerve head size was associated with the highest achieved level of education and time needed to perform a standardized perimetric test. Considering level of education and time needed to perform perimetry as surrogates of cognitive function, optic nerve head size was related to cognitive function.

• T062 / 2727

**Spectral analysis of ocular pulse amplitude recordings obtained using a contact lens sensor**

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**Purpose** The aim of this study was to investigate whether the ocular pulse amplitude (OPA) can be detected in fluctuation curves recorded with the Sensimed Triggerfish® Sensor (TS), and analysed by Fast Fourier Transform (FFT).

**Methods** 40 subjects (20 open angle glaucoma [OAG], 20 healthy) underwent one hour of TS monitoring. Intraocular pressure and OPA were measured by dynamic contour tonometry (DCT) and DCT OPA was used as the reference. Segments of TS OPA fluctuations were visually identified (VI), and the amplitude (mVequ) was measured (VI-TS OPA). In addition, FFT signal analysis, which reduces a periodic function into its frequency components, was performed on VI segments, and the amplitude (arbitrary units [AU]) of the fundamental measured (FFT-TS OPA). The correlation between VI- and FFT-TS OPA was investigated. VI- and FFT-TS OPA were compared with DCT OPA.

**Results** In 3 subjects, TS recording failed. In 8 subjects, either no OPA or no fundamental could be identified. For the remaining 29 subjects (13 OAG, 16 healthy), both mean ( $\pm$ standard deviation) VI- and FFT-TS OPA were lower in OAG ( $9.0\pm3.9$ mVequ and  $184.6\pm303.7$ AU) than in healthy subjects ( $10.2\pm4.5$ mVequ and  $303.3\pm292.0$ AU). Mean DCT OPA was higher in OAG ( $2.7\pm0.8$ mmHg) than in healthy subjects ( $2.3\pm0.6$ mmHg) ( $p>0.05$  for all). There was a significant correlation between VI- and FFT-TS OPA (Spearman's rho=0.84;  $p<0.0001$ ). In OAG subjects only, the correlation between VI-TS OPA and DCT OPA (Spearman's rho=0.52,  $p=0.072$ ) and FFT-TS OPA and DCT OPA (Spearman's rho=0.48,  $p=0.099$ ) approached significance.

**Conclusion** It is possible to identify OPA in TS fluctuation curves and to analyse OPA by FFT. There is a weak association between TS OPA and DCT OPA in OAG.

• T063

**Correlation between retinal ganglion cell count and morpho-functional parameters in glaucoma**

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**Purpose** To evaluate the correlation between retinal ganglion cell (RGC) count and morpho-functional parameters provided by spectral-domain optical coherence tomography (SD-OCT) and standard automated perimetry (SAP)

**Methods** 52 eyes evaluated for open angle glaucoma underwent a complete ophthalmologic examination including SAP and SD-OCT evaluation of retinal nerve fiber layer (RNFL) thickness and macular ganglion cell complex (GCC). RGC counts were obtained using a previously derived algorithm proposed by Medeiros et al. Patients were divided into 3 groups according to glaucoma severity staged by Glaucoma Staging System 2: suspects (stage 0-border), early (stage 1-2) and manifest glaucoma (stage 3-4-5)

**Results** Average RGC count estimate was  $933803\pm132506$  in suspects,  $685583\pm158345$  in early and  $385316\pm185374$  in manifest glaucoma ( $p<0.001$ ). The same trend was noticed for the considered parameters, even if the major loss was found between early and manifest glaucoma in functional data (MD and PSD) and between suspects and initial glaucoma in structural data (RNFL and GCC). RGC count showed very poor correlation with PSD in all groups ( $r<0.3$ ), a progressively increased correlation with MD according to glaucoma severity ( $r>0.3$  in suspects,  $r>0.9$  in manifest glaucoma), a moderate correlation with RNFL ( $r>0.6$ ) and GCC ( $r>0.5$ ) in all groups

**Conclusion** A substantial loss of RGCs is detectable in all groups and proportional to glaucoma stages. Structural evaluation of optic nerve (RNFL) and inner macular retina (GCC) can provide a useful diagnostic tool in all stages while SAP is well correlated only to substantial loss of RGC. Macular RGC are involved in early glaucoma loss

• T064

**Progression of visual field in patients with primary open-angle glaucoma (2): Level of progression**

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**Purpose** To compare the visual field rate (VF) of progression of non evolutive and evolutive patients with treated ocular hypertension (OHT) or primary-open angle glaucoma (POAG).

**Methods** From a multicentric database, 441 eyes of 228 patients with treated OHT or POAG followed up at least 6 years with Humphrey 24.2 Sita-Standard VF exam at least twice a year were identified and classified in 5 groups: 104 OHT, 205 early glaucoma ( $MD>-6$ dB), 45 moderate glaucoma ( $MD$ -6 to  $-12$ dB), 41 advanced glaucoma ( $MD$ -12 to  $-18$ dB) and 46 severe glaucoma ( $MD$ < $-18$ dB). With final data, patients were classified in 2 sub groups: "evolutive" or "stable" patients if worsening or not to upper grade. Rate of progression during follow-up period was calculated using the trend analysis of the Guided Progression Analysis software.

**Results** The mean follow-up was 8.4 years. We found respectively for "stable" and "evolutive" sub-groups, following rates of progression :  $MD = -0.01$  and  $-0.13$ dB/year ( $-0.03$  &  $-0.41$ %VFI/year) in eyes with OHT,  $-0.07$  &  $-0.80$ dB/year ( $-0.23$  &  $-2.39$ %VFI/year) in early glaucoma group,  $-0.02$  &  $-0.91$  dB/year ( $-0.46$  &  $-3.04$ %VFI/year) in moderate glaucoma,  $-0.21$  &  $-0.82$ dB/year ( $-1.13$  &  $-3.57$ %VFI/year) in advanced glaucoma.

**Conclusion** In early to advanced stages of glaucoma, "stable" patients showed a low rate of progression, compared to "evolutive" subgroup with a common rate around  $-0.80$ dB/year. These values could represent a landmark of evolution in glaucoma.

**• T065****Ocular Pulse Amplitude is associated with ocular blood flow velocities in healthy subjects and patients with primary open angle glaucoma, but not normal tension glaucoma**

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**Purpose** Purpose: to determine the correlation between ocular blood flow changes throughout the cardiac cycle on ocular pulse amplitude (OPA) in glaucoma patients and healthy subjects.

**Methods** Method: A prospective, case-control study was designed with dynamic contour tonometry and color Doppler Imaging performed in healthy controls and glaucoma patients. An offline waveform analysis of the Doppler signal was performed by a masked observer for each of the retrobulbar arteries. Linear regression models using intraocular pressure (IOP), age and Doppler waveform variables [(resistance index (RI) and systolic/diastolic velocity ratio (Sm/Dm)] were designed to identify independent variables associated with OPA.

**Results** Results: 192 patients were included [healthy controls: 55; normal tension glaucoma (NTG): 63, primary open-angle glaucoma (POAG): 74]. OPA showed a significant associated with the constructed model in healthy individuals and in POAG patients ( $r=0.54$ ,  $p<0.001$ ;  $r=0.43$ ,  $p<0.001$ , respectively). Short ciliary arteries flow variables were the only vascular-related parameters independently associated with OPA in these groups ( $p<0.05$ ). IOP was independently associated with OPA in the POAG group ( $p<0.001$ ), but not the healthy group ( $p=0.48$ ). OPA in the NTG group did not correlate with the constructed model ( $r=0.24$ ,  $p=0.21$ ).

**Conclusion** Conclusions: OPA seems to reflect blood flow changes in the short ciliary arteries in healthy individuals and POAG patients, but not in NTG patients. In the latter, the ocular pulse may relate to other variables than blood flow velocities.

**• T066 / 3727****Motion perception in early glaucoma**

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**Purpose** The aim was to underline changes in the motion perception for early glaucoma. Our hypothesis consisted in inquiring if the impairment of the magnocellular pathway may modify the motion perception.

**Methods** We included 14 healthy subjects and 14 patients with early primary open angle glaucoma. A moving target was presented on a semicircular screen; participants were asked to localize the Ending Point (EP) of each motion. A stimulus consisted in a white dot moving horizontally. Two different laws of motion were displayed: a "biological" motion, consisting in a bell-shaped velocity profile, and a "non-biological" motion corresponding to a constant velocity profile. EP may be displayed in the pericentral or peripheral visual field. The experiment was constituted by 192 trials. We calculated the PCE (Position Constant Error) which was defined as the average difference between the estimation of the EP indicated by the participant versus the true location of the target. The PVE (Position Variable Error) was defined as the standard deviation of the responses of each participant.

**Results** All the participants overestimated the EP ( $13.23\pm8.87$  mm for healthy subjects and  $17.06\pm13.07$  mm for glaucoma patients,  $p=0.2518$ ). The PVE was  $28.14\pm6.86$  mm for healthy subjects and  $40.95\pm9.83$  mm for glaucoma patients ( $p=0.0004$ ). There was a significant difference in the PVE for both groups when stimulus moved accordingly to the "non-biological" velocity profile ( $p=0.0001$ ). Glaucoma patients had substantial PVE increase when the target image had a "non-biological" speed profile ( $p=0.0388$ ).

**Conclusion** The unexpected lack of difference between normal subjects and patients in localizing a moving stimulus suggested that the visual deficiency could be partly compensated by endogenous informations.

**• T067 / 4228****Validation of Testvision, an internet-based test for the detection of visual field loss**

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**Purpose** The aim of the present study was to determine if the program Damato Testvision (DT), [www.testvision.org](http://www.testvision.org), adequately detects visual field loss from glaucoma.

**Methods** DT is available in three versions and was tested on 97 patients (188 eyes). The procedure was performed with a laptop connected to a computer monitor and a mouse placed on a height adjustable table. To determine sensitivity and specificity DT was compared to two gold standards: 1; Hodapp, Parrish and Anderson classification (Hodapp) of Humphrey 30:2 and 2; doctors' diagnoses.

**Results** A total of 361 DT Standard tests were performed on 115 eyes. DT comparisons with Hodapp achieved sensitivity and specificity of 55% and 93% respectively when comparing no disease to early glaucomatous loss (AUC; 0.871). If early loss was merged with the no disease group, the sensitivity and specificity were 78% and 90% respectively (AUC; 0.931) Compared to the doctors' diagnosis DT achieved sensitivity and specificity of 52%; 96% respectively when borderline patients were classified in the glaucoma group (AUC; 0.867). If borderline patients were classified as controls: DT achieved sensitivity and specificity of 63%; 92% (AUC; 0.897).

**Conclusion** Our study indicates that DT may be a promising tool to detect visual field defects in a pre-selected population. The test is successful without prior training of the test person but minimal computer knowledge is advisable. The authors believe that internet based visual field examination may play a role as an easy accessible and affordable test. With further development, DT could be a tool for glaucoma screening in the home setting. Future studies need to evaluate DTs performance in the general population without prior selection.

**• T068****Primary health care model for detection of ophthalmological visual disturbances in a population of employees of Universidad de Tarapaca, Chile**

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**Purpose** Implement a primary health care model for the Universidad de Tarapacá personnel in Arica, Chile, which is a desert region.

**Methods** The model was applied at 510 employees and consisted of applying a survey and eye exams such as: Visual acuity for near and far, autorefractometria, biomicroscopy, IOP. In the analysis measures of central tendency, proportions, means and standard deviation were used. The ratio differences were analyzed in bivariate statistic chi2 and T Student.

**Results** The average age was 48 years old, 57% female and 43% male. The 27% have Aymara ancestry and of these, 13% have ocular disorders. The 18% have systemic diseases and 73% have just one family member with an ocular disease. One of every 4 states to have a diagnosed eye disease. The 50% of the total has 100% of AV without optical devices. The 34% use optical lenses that do not alter their visual acuity. Of those over 40 years old, 3% has IOP above 24 mm Hg, 7% over 21 and 90% less than 21. The 16% has Pterigion symptomatic and 5% are bilateral. The 61% of the total study population reports to feel the need to be assisted an eye professional.

**Conclusion** It is important to take measures for public health action and modify the regional and national public policies of our country through implementation models resolute primary eye care for improving the access to healthcare systems. This makes better use of the specialized medical resources.

**• T069****Distribution of ocular biometric parameters during the eye growth**

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**Purpose** To determine the distribution of ocular biometric parameters and their correlation with age and gender during the eye growth.

**Methods** In a cross-sectional study, a multi-stage stratified cluster sampling was used to sample the study population from Dezful schools, a city in the southwest of Iran. Participants were selected from the all school grades and both genders. Examinations were performed after obtaining consent from the subjects' parent. LENSTAR/BioGraph (WaveLight AG, Erlangen, Germany) was used for ocular biometry.

**Results** Of 864 selected subjects, 683 participated in the study (response rate: 79.1%); 377 participants (55.2%) were males. Mean age of the subjects was  $12 \pm 3.4$  years (range: 6.0-18 years). Mean and 95% confidence interval(CI) of axial length (AL), anterior chamber depth (ACD), lens thickness (LT), central corneal thickness (CCT), corneal diameter (CD), and pupillary diameter(PD) were 23.13 mm (22.93-23.33), 3.01 mm (2.96-3.06), 3.58 mm (3.55-3.61), 549.33 mic (546.59-552.07), 12.34 mm (12.31-12.38), and 4.97 (4.91-5.03), respectively. Ocular biometric parameters were normally distributed. Distribution of LT and CD were leptokurtic and the distribution of the PD was platykurtic. The mean of AL, ACD, CD and CR were significantly higher in boys and the mean of LT was significantly higher in girls. Axial length and ACD increased and the LT decreased significantly with age. Axial length and CR had the highest correlation ( $r=0.669$ ) and ACD had the strongest correlation with the

**Conclusion** The results of this study indicated that AL and ACD were intermediate and shorter respectively. Corneal diameter was bigger in comparison with the other studies. The majority of the changes were observed in AL and LT.

**• T071****Profiling miRNAs in a hyperglycemic and hypoinsulinemic Ins2Akita mouse model**

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**Purpose** Diabetic retinopathy (DR) is a leading cause of blindness. Our goal is to identify novel genetic targets involved in DR. microRNAs (miRNAs) are non-coding RNAs that regulate gene expression. The purpose of this study is to characterize the in vivo perturbations of retinal miRNAs in a hyperglycemic and hypoinsulinemic mouse model. Here, we characterize the vascular and macroscopic anatomic properties of the Ins2Akita mouse and then profiled the miRNA abundance longitudinally, using a cost effective multiplexed next generation sequencing method.

**Methods** Heterozygous male Ins2Akita with a mutation in the insulin 2 gene, were age matched with C57BL/6J mice. We analyzed: vascular permeability by Evans blue extravasation, performed fundus photography and fluorescein angiography, and surveyed for functional visual impairment by electroretinography (ERG). Then, we measured miRNA abundance in the retina and choroid/retinal pigmented epithelium (RPE) at several time points using multiplexed libraries of Ins2Akita and wild-type mice at 1 and 3 months of age.

**Results** Morphological changes and fluorescein angiogram abnormalities were identified in the retinas of Ins2Akita at 1 and 3 months. We observed substantially increased vascular permeability in Ins2Aktia retina at 3 months. Functional changes were observed by ERG at 6 months of age. miRNA profiles were generated for Ins2Akita and control mice (1 and 3 months), which showed substantial down regulation of several miRNAs including miR-126 and miR-143 in the choroid/RPE, when compared to control.

**Conclusion** miRNAs identified offer potential targets for genetic modulation in hyperglycemic induced pathology in the eye

**• T070****Eye problems in deaf children: A case-control study**

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**Purpose** To compare the prevalence of refractive errors and amblyopia between deaf and normal children

**Methods** In this case control study, cases were selected from deaf school children in Mashhad. The control group consisted of children with no hearing problem. The sampling was done utilizing the cluster sampling method. All of the samples underwent refraction, cover test and visual examinations.

**Results** 254 children in case group and 506 children in control group were assessed. The mean spherical equivalent was  $1.7 \pm 1.9$  D in case group, which was significantly different from the control group ( $0.2 \pm 1.5$ ) ( $p < 0.001$ ). The prevalence of hyperopia was 57.15 and 21.5% in case and control groups, respectively. But myopia was mostly seen in the control group ( $p = 0.007$ ). Mean cylinder was  $0.65 \pm 1.3$  D and  $0.43 \pm 0.62$  D in case and control groups, respectively. Logistic regression showed that the chance of Amblyopia was significantly greater in cases.

**Conclusion** Comparing with children in the same ages, deaf children have much more eye problems; therefore there must be a possible relation between deafness and eye problems. Paying attention to assessing eye health in deaf children, may prevent them from adding eye problems to deaf difficulties.

**• T072****Visual function changes in an animal model of retinitis pigmentosa**

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**Purpose** To evaluate visual acuity and contrast sensitivity in pigmented P23H-1 rats.

**Methods** The assessment of visual parameters was carried out using OptoMotry® system, CerebralMechanics Inc. Visual acuity and contrast sensitivity curve were calculated. Eight pigmented heterozygous P23H-1 rats were measured at 30, 60, 90, 120, 150, 180, 210 and 240 postnatal days. Visual function of Long Evans rats was evaluated to provide a background for the study.

**Results** Age-related losses in visual acuity were found: from 0.500 cyc/deg (P30) to 0.069 cyc/deg (P240). The same trend was observed in contrast sensitivity curve. Peaks from 54.56 (P30) to 1.00 (P210 and P240) were obtained for a spatial frequency of 0.089 cycles/degree. Both parameters began to decline at 30 days of age and continued until P240, when rats were euthanized for retinal immunohistochemistry. Visual acuity of Long Evans rats was close to 0.540 cyc/deg. Contrast sensitivity curve displayed a typical inverted-U shape.

**Conclusion** Visual acuity and contrast sensitivity decrease while increasing age of the P23H-1 rats. Similar results to control animals were found at P30. However, a gradual decrease in visual acuity and contrast sensitivity was observed from near normal at P30 to low levels at P240. It agrees with the progressive loss of photoreceptors.

• T073 / 4676

**Influence of refractive error and axial length on retinal vessel geometric characteristics**

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**Purpose** To evaluate the influence of refractive error and axial length (AL) on retinal vascular network geometry measurements in an adult Asian population.

**Methods** This was a population-based, cross-sectional study on 2882 persons with diabetes in the Singapore Malay Eye Study (SiMES). Spherical equivalent refraction (SE) was assessed using an autokeratometer and subjective refraction. AL was measured by IOL Master. Retinal vascular caliber, tortuosity, and branching characteristics were quantified from retinal fundus photographs using a semi-automated computer-assisted program according to a standardized protocol.

**Results** In multivariate analyses adjusting for age, gender, education, smoking, blood pressure, diabetes status, and anti-hypertensive medication use, longer AL and more myopic refraction were associated with narrower retinal arterioles and venules.( $p \leq 0.001$  for all) and less tortuous (straighter) arterioles.( $p < 0.001$  for both). Longer AL and more myopic refraction were also associated with increased branching coefficients in both arterioles ( $p < 0.001$  for both) and venules.( $p = 0.02$  and  $p < 0.001$  respectively). Longer AL and more myopic refraction were associated with more acute branching angles in arterioles ( $p < 0.001$  for both) but not venules.

**Conclusion** Myopic refractive errors and longer AL are associated with narrower retinal arterioles and venules, less tortuous arterioles, and increased branching coefficients in both arterioles and venules. These findings provide insights into ocular blood flow in myopia, and also suggest that future studies evaluating these retinal parameters should account for the influence of AL and refractive error.

• T074 / 4677

**Early dietary therapy in preventing progression of retinopathy in long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency caused by the homozygous G1528C mutation**

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**Purpose** To evaluate the efficacy of the early start of a low fat, high carbohydrate diet in preventing progression of pigmentary retinopathy, a characteristic feature of long-chain 3-hydroxy-acyl-CoA dehydrogenase (LCHAD) deficiency, a mitochondrial long-chain fatty acid  $\beta$ -oxidation defect.

**Methods** 13 Finnish patients with early diagnosis of LCHAD deficiency caused by the homozygous G1528C/Q510Q common mutation, were followed up to 20 years (median 6 y). An ophthalmic examination was performed every 1-2 years including BCVA, colour vision, axial length, fundus colour photography, OCT, and ERG.

**Results** 11 out of 13 patients had mild pigmentary changes already in their first ocular exam (median age of 6 mos), while 2 had pale newborn retina. The initial fundus changes were most often pigment granularity in the posterior pole, hyperpigmented macula and pale fundus. In 12 patients, with good compliance of the dietary therapy, the pigmentary changes progressed only mildly or not at all, remaining at stage II of retinopathy with no atrophy and visual compromise. The only patient with an overall poor outcome, had progression of the chorioretinopathy to stage III, presenting atrophy in the posterior pole with relative sparing of the macula and far periphery.

**Conclusion** Our long-term ocular follow-up data are promising and suggest that the low-fat, high-carbohydrate diet started during the first months of life may delay or even halt the progression of the pigmentary retinopathy and possibly prevents the visual handicap in LCHAD deficiency.

• T075

**A cone-rod dystrophy patient with a homozygous RP1L1 mutation**

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**Purpose** To describe a family with a cone-rod dystrophy patient who has homozygous mutation of the RP1-like protein 1 (RP1L1) gene.

**Methods** A family including a cone-rod dystrophy patient underwent detailed ophthalmic clinical evaluations including high resolution cone photoreceptor imaging with adaptive optics fundus camera. Mutation screening of the sequence of RP1L1 gene were performed by DNA sequencing analysis in this family members

**Results** A patient showed a mild reduction of cone and flicker response in full-field electroretinogram (ERG). Her ERG also showed slight decrease in the amplitude of rod response. IS/OS junction and COST line in SD-OCT images are severely disturbed. Response of multifocal ERGs (mfERGs) of the patient was severely reduced. A homozygous RP1L1 mutation (c.3628 T>C) was identified in the patient. The mutation c.3628 T>C in exon 4 resulted in the substitution of proline for serine at amino acid position 1210. This mutation was not reported in SNP database. The serine at position 1210 is well conserved among the RP1L1 family in other species. Four out of five computational assessment tools predicted that this mutation is damaging to the protein function. This mutation was not present in 460 control alleles. Family members with heterozygous S1210P mutation showed normal best-corrected visual acuity (BCVA), SD-OCT, mfERGs, and focal macular ERGs. Adaptive Optics (AO) images of the patient showed severe reduction of cone density and irregular cone mosaic despite family members with heterozygous S1210P mutation showed normal cone density and regular cone mosaic.

**Conclusion** We have demonstrated a possibility that autosomal recessive cone-rod dystrophy may be caused by homozygous RP1L1 mutation.

• T076

**Hunter Syndrome after 12 months of enzyme replacement treatment: A case report**

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**Purpose** Hunter syndrome (mucopolysaccharidosis type II) is a rare X-linked-recessive inherited disease caused by deficiency of the lysosomal enzyme iduronate-2-sulfate leading to accumulation of dermatan-sulfate (DS) and heparan sulfate (HS) in nearly all cell types. Described ophthalmic manifestations include exophthalmos, hypertelorism, pigmentary retinopathy, epiretinal membranes, scleral thickening, optic disc oedema and maculopathy. Currently, enzyme replacement therapy (ERT) with recombinant human iduronate-2-sulphatase (idursulfase) is the treatment of choice. We present the case of a 26-year-old patient with Hunter syndrome after 12 months with ERT.

**Methods** Case report of a 26-year-old patient diagnosed with Hunter syndrome, treated with idursulfate during one year.

**Results** After 12 months with ERT the visual acuity, visual field study, ERG and funduscopic findings remained stable.

**Conclusion** Hunter syndrome is a severe multisystemic disease. ERT is an important therapy that can help improve, or stabilize, patients' symptoms.

• T077

**Interleukin-8 promoter polymorphism is associated with the initial response to bevacizumab in AMD treatment**

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**Purpose** To study the association of potential key single-nucleotide polymorphisms with the short-term anatomic response to bevacizumab treatment in exudative age-related macular degeneration (AMD).

**Methods** Clinical data of 96 bevacizumab-treated exudative age-related macular degeneration patients were analyzed retrospectively. Blood DNA was collected. Based on the disappearance of intra- or subretinal fluid in optical coherence tomography, patients were graded as responders, partial responders, or nonresponders after 3 initial treatment visits and a median time of 3.5 months. Representative single-nucleotide polymorphisms of interleukin 8, vascular endothelial growth factor, erythropoietin, complement factor H, complement component C3, and LOC387715 genes were analysed.

**Results** Interleukin 8 promoter polymorphism -251A/T, conferring a more active interleukin-8 system, was significantly associated with persisting fluid in optical coherence tomography. The A allele was more frequent in nonresponders than in responders ( $P = 0.033$ ). In multivariate modeling, the AA genotype of -251A/T ( $P = 0.043$ ) and occult ( $P = 0.042$ ) or predominantly classic ( $P = 0.040$ ) lesions predicted a poorer outcome.

**Conclusion** The interleukin -8 pathway may modulate the early anatomic response to anti-VEGF treatment in AMD. A possible activation of interleukin -8 production in patients may represent a compensatory mechanism to chronic blockade of VEGF signalling in AMD lesions

• T078

**Clinical findings in a Roma family with autosomal dominant cone-rod dystrophy**

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**Purpose** To make a clinical assessment of patients affected by autosomal dominant cone-rod dystrophy (adCORD).

**Methods** A three-generation autosomal dominant pedigree of Romani origin with non syndromic CORD was identified. Eight affected and 14 unaffected individuals were clinically ascertained. All affected relatives were studied. Clinical evaluation included best corrected visual acuity determination, funduscopy, Humphrey perimetry, Farnsworth Hue-28 color testing, fluorescein angiography, and full-field electroretinogram (ERG).

**Results** All affected individuals presented reduced visual acuity (0.01 - 0.4) and photophobia with slightly variable but early age of onset (around 13 years of age). Funduscopic examination and fluorescein angiography revealed advanced changes including bone spicule-like pigment deposits in the midperiphery and macular area along with retinal atrophy, narrowing of the vessels, and waxy optic discs. Visual fields demonstrated central scotoma. Electrophysiologic examination of the patients detected an abnormal cone-rod ERG (20-30 $\mu$ V) with photopic amplitudes more markedly reduced than the scotopic. Flicker responses were missing and Farnsworth Hue-28 test found protanopia.

**Conclusion** In this study we report a family of Gypsy origin affected by autosomal dominant cone-rod dystrophy. Identification of the disease causing gene may eventually contribute to new knowledge on the pathogenesis of this condition.

• T079

**Examination of colour vision deficiency in different types retinitis pigmentosa**

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**Purpose** Investigation of the colour vision deficiency in different types of retinitis pigmentosa (RP): autosomal dominant, autosomal recessive, X-linked recessive, and isolated cases.

**Methods** Colour vision investigation was conducted on 42 patients (18 women, 24 men) with RP using the Farnsworth 28 HUE-test, anomaloscope -IF-2 Tomay, flicker threshold test, standard pseudoisochromatic table and Tritan Lantone album. The age of the patients varied from 5 to 68 years (mean  $39 \pm 17$ , SD).

**Results** In 73.3 % of patients with RP we established acquired colour vision deficiency (dyschromatopsia). Among the patients with acquired colour vision deficiency we found out using the above-mentioned tests that 67.8% were with tritanomaly, 19.3% were with manifested deuteranomaly and 12.9 % were with protanomaly. The type of dyschromatopsia does not show any relation to the age and visual acuity of patients. When no foveal lesion was apparent in patients with visual acuity better than 20/40, patients with autosomal dominant disease showed superior performance on color vision testing when compared to autosomal recessive, X-linked recessive, and isolated cases. The Farnsworth 28 HUE test was more sensitive in detecting poor color vision performance than the anomaloscope -IF-2 Tomay.

**Conclusion** We established a great percentile of patients with RP suffer from colour vision deficiency. Our study reveals that colour vision investigation has an important role in PR diagnosis.

• T080

**Retigabine-induced retinal dystrophy: First reported case**

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**Purpose** A recent FDA warning reported that retigabine (ezogabine in the US), a third-generation antiepileptic drug introduced in 2011 for the management of refractory epilepsy, can cause blue skin discoloration and retinal pigmentary changes. Our purpose is to present the first documented case of retinal dystrophy secondary to this drug.

**Methods** This case report shows the characteristics of the so-called "blue person syndrome," such as blue pigmentation around lips, fingernails and toenails. Likewise, the patient developed a bilateral and asymptomatic retinal dystrophy confirmed by color and red-free fundus photographs.

**Results** A 53-year-old woman noted blue pigmentation around lips, fingernails and toenails for 6 months. She suffered from refractory epilepsy and underwent 1200 mg daily of retigabine (Trobalt<sup>®</sup>) for 6 years. BCVA was 20/20 bilaterally. Slit-lamp examination showed no conjunctival discoloration. IOP, ocular motility, pupillary reactions and color vision test were normal. Ophthalmoscopy revealed bilateral retinal pigmentary changes running outward from the optic disk, with an appearance similar to that seen in angioid streaks. Several grey, fine subretinal branching bands radiated out from an area of peripapillary pigment alteration. Red-free fundus photographs showed, along the temporal vascular arcades, a thinned and depigmented retina, allowing visualization of underlying choroidal vessels. Visual field testing, fluorescein angiography, autofluorescence and SD-OCT were unremarkable.

**Conclusion** Ophthalmologists and neurologists should be aware of the possibility of ophthalmological toxic effects in patients taking retigabine. When such effects are detected, the drug should be discontinued.

## • T081

**Alu retrotransposon quantification in the retina and plasma: Mechanism-based risk assessment in age-related macular degeneration**

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**Purpose** Alu RNAs are endogenous pathogens in age-related macular degeneration (AMD). Currently, efforts to develop diagnostic and prognostic tools are limited. Yet, because AMD is a chronic disease, tools to detect pre-clinical disease and monitor progression are an essential part of an effective and comprehensive therapeutic strategy. We tested plasma Alu RNA as a potential biomarker for AMD. Also, cells can convert Alu RNA into DNA, and as such, we determined Alu DNA content of RPE cells. We also genotyped RPE cells for a panel of known AMD risk single nucleotide polymorphisms, and developed an AMD risk stratification system based on age, genotype, and Alu DNA content.

**Methods** RPE cells were isolated from human donor eyes were harvested within 5-10 hours after death. RPE cells were grown in cell culture for four passages. DNA was extracted and subjected to genotyping for AMD-associated SNPs using TaqMan probes. Plasma sample RNA was extracted with Trizol LS and reverse transcribed. qPCR for DNA performed with standard curves of Alu-specific primers and housekeeping single copy genes; for cDNA, Alu quantified per mL plasma.

**Results** The RPE of AMD donors contain increased quantities of Alu DNA. Our results suggest that Alu DNA content increases as a function of age and disease status, and that genotype also modulates disease risk. Finally, Alu RNA content in plasma was significantly increased in AMD versus age-matched healthy controls.

**Conclusion** These efforts set the stage for the utilization of plasma Alu RNA quantity as a novel mechanism-based biomarker in AMD, and also implicate increased Alu DNA burden as a potential mediator of Alu RNA-induced RPE cell death in AMD.

## • T082

**Angiopoietin-like protein 2 contributes to pathogenesis of diabetic retinopathy**

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**Purpose** Recent studies show that angiopoietin-like protein 2 (Angptl2) contributes to chronic inflammation in diabetes tumor, rheumatoid arthritis and dermatomyositis, Angptl2 is expressed in endothelial cells, and increased by hypoxia and endoplasmic reticulum stress in HUVEC, and serum level of Angptl2 is increased in diabetes. However, the function of Angptl2 in diabetic retinopathy is still unknown. In this study, we investigate the Angptl2 concentration in vitreous fluid and expression in fibrovascular membrane of PDR patients to evaluate the role of Angptl2 in pathogenesis of diabetic retinopathy.

**Methods** We collected vitreous samples at the time of vitreous surgery from 33 PDR and 29 nondiabetic patients. The concentration of Angptl2 in vitreous samples was measured by using the human Angptl2 enzyme-linked immunosorbent assay, and we examined Angptl2 protein expression in fibrovascular membrane from PDR patients by immunohistochemistry using both anti-Angptl2 and anti-CD31 or anti-Mac2 antibody. Next, we examined Angptl2 expression under the hypoxic condition, and ICAM-1 expression induced by Angptl2 in human retinal endothelial cells (HRECs).

**Results** The vitreous concentration of Angptl2 significantly increased in patients with PDR compared with controls ( $P < .001$ ). Double immunofluorescent staining showed that Aangptl2 was expressed in endothelial cells and macrophages in fibrovascular membrane. In vitro study, we found that Angptl2 expression was increased under 2% hypoxic condition in HRECs, and Angptl2 promoted ICAM-1 expression in HREC through the activation of NFkB signaling.

**Conclusion** Aangptl2 may play an important role in the pathology of diabetic retinopathy by causing the chronic inflammation in retinal endothelial cells.

## • T083

**Photoreceptor integrity and visual acuity in retinitis pigmentosa**

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**Purpose** To assess the correlation between the status of the inner segment/outer segment junction (IS/OS) and visual acuity in retinitis pigmentosa.

**Methods** One hundred and ninety-six eyes of 100 patients with RP were examined with the spectral optical coherence tomography (3D-OCT-2000). The status of the inner segment/outer segment junction (IS/OS) (Grade 1: absent, Grade 2: blurred discontinuous, or Grade 3: distinct) was evaluated. Correlation between visual acuity and each of the measurements were examined.

**Results** Of the 196 eyes, IS/OS was absent in 61 eyes (30.9%), blurred in 71 eyes (36.36%) and distinct in 64 eyes (32.74%). Total retinal thickness was not correlated with visual acuity. The mean logMAR visual acuity (VA) in the IS/OS absent group ( $0.65 \pm 0.12$ ) was worse than the one seen in the IS/OS blurred ( $0.23 \pm 0.41$ ) or IS/OS distinct groups ( $0.08 \pm 0.44$ ) ( $P < 0.001$ ). But eighteen per cent of the eyes with a normal foveal IS/OS line, normal COST line and normal perifoveal layers have a bad visual acuity. While seven per cent of eyes with absent foveal IS/OS line have surprisingly a good visual acuity.

**Conclusion** The status of photoreceptor IS/OS line is an important parameter that needs to be examined in RP patients, while perifoveal retinal layers need not be examined. The presence of the foveal photoreceptor IS/OS line is associated with better visual acuity and thicker fovea in RP patients. But, in a few cases, the foveal photoreceptor IS/OS line can be absent with a good visual acuity.

## • T084

**DNA methyltransferases are differentially expressed in human eyes**

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**Purpose** We studied DNA methyltransferases (DNMT) implicated in CpG methylation pattern. The aim of our work was to extensively check the presence of DNMT (mRNA and protein levels) and quantify their expressions in the anterior segment.

**Methods** mRNAs were extracted from human cornea, conjunctiva, capsule, trabeculum and from related cell lines (HCE, HTCE, CHANG and TM). cDNAs were generated by reverse transcription, quantified by amplification with specific primers for DNMT1, 3A, 3B and 3L and normalized to the housekeeping gene 36B4. For immunohistochemistry, normal human corneas were provided by the regional corneal graft bank. All proteins were detected by specific primary antibody for DNMT1, 3A, 3B and 3L (Santa-Cruz<sup>®</sup>), negative control were done with a rabbit IgG antibody as primary one. Secondary antibody Cy3-coupled was used.

**Results** All DNMT are expressed in the human eyes but at different levels. DNMT3L looks like to be the weakly expressed. All tested cell lines also expressed DNMTs. Quantitative RT-PCR demonstrated strong differences between members and zones of expression of these DNMTs. Our transcripts results were confirmed by immunohistochemistry. All tested cell lines also expressed DNMTs. Quantitative RT-PCR demonstrated strong differences between members and zones of expression of these DNA methyltransferases. Our transcripts results were confirmed by immunohistochemistry experiments on cornea sections.

**Conclusion** For the first time, an inventory of the DNMT expression in tissues and cells lines from human ocular anterior segment was performed. Future studies will certainly permit to demonstrate the presence of a direct link between a deregulation of the CpG methylation pattern and the already "well known" eye pathologies.

• T085 / 2767

**Analysis of mitochondrial sequences in patients with keratoconus**

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**Purpose** Keratoconus (KTCN) is thinning and anterior protrusion of the cornea. The etiology of KTCN remains unknown. Both genetic and environmental factors are associated with the disorder. The purpose of this study was to identify novel genetic factors by analyzing mitochondrial sequences in cases and controls from Polish population. There are available only a few analyses of some mitochondrial sequences in KTCN studies.

**Methods** A total of 96 individuals from Polish population were included into this study. Chosen mtDNA fragments of all individuals were sequenced.

**Results** Sequencing analysis of chosen mitochondrial genome fragments have revealed numerous alterations including several novel polymorphisms. No sequence variants segregated significantly more frequent with KTCN have been identified.

**Conclusion** Analysis of chosen fragments of mitochondrial genome in Polish patients have revealed numerous sequence variants, however our results do not support involvement of mtDNA changes in KTCN in Polish patients. The KTCN development does not depend on a single change in the gene, but on the accumulation of numerous sequence variants. The complexity of the genetic basis of KTCN causes the need to find another approach to further investigate the etiology of KTCN. Support: National Science Centre (Poland), Grant 2011/03/N/NZ5/01470

• T086 / 2766

**Genotype-phenotype correlation in two patients with posterior polymorphous corneal dystrophy 3**

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**Purpose** To determine the molecular genetic cause of posterior polymorphous corneal dystrophy (PPCD) in four Czech probands.

**Methods** Extensive ophthalmological examination including Pentacam and specular microscopy, and direct sequencing of the ZEB1 coding region were performed.

**Results** Two novel frameshift mutations within ZEB1 were identified: c.2617dup in exon 8 in a 21-year-old female, considered to be most likely de novo in origin, and c.698dup in exon 6 in a 21-year-old male. The first case had mild changes consistent with a PPCD diagnosis and best corrected visual acuity (BCVA) bilaterally was 1.0. The corneal phenotype of the second case was more severe with a BCVA of 0.4 in the right and 0.05 in the left eye. Corneas of both probands were abnormally steep (keratometry readings K1  $\geq$  47.4 D and K2 45  $\geq$  49.2 D) with increased pachymetry values, but no pattern indicative of keratoconus. Specular microscopy in both patients revealed reduced endothelial cell density (range 1055- 655 per mm<sup>2</sup>). Both probands had a history of surgery for inguinal hernia; the male patient also reported the presence of hydrocele.

**Conclusion** Nucleotide changes within the coding region of ZEB1 underlie the pathogenesis of PPCD in 4 out of 23 (17.4%) Czech probands.

• T087

**Association between endothelin receptor type A gene polymorphisms EDNRA (C+70G, C+1222T) and the endothelin-1 plasma concentration in patients with normal tension glaucoma**

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**Purpose** The purpose of his study was to determine the prevalence of endothelin receptor type A gene polymorphisms EDNRA (C+70G, C+1222T) and its correlation to the plasma concentration of endothelin-1 (ET-1) in patients with normal tension glaucoma (NTG).

**Methods** The study enrolled 36 NTG patients including 23 women and 13 men. In order to analyze the frequency of polymorphic variants of endothelin receptor type A genes EDNRA (C+70G, C+1222T), DNA was isolated from peripheral blood and SNP (Single Nucleotide Polymorphism) genotyping was performed using RT-PCR method (Real-Time Polymerase Chain Reaction). The plasma ET-1 concentration was detected using an enzyme immunoassay.

**Results** Analysis of the prevalence of endothelin receptor type A gene polymorphisms EDNRA C+70G was: GG-30.5%, CC-14.0%, CG- 55.5%. The frequency of the genotype GG tended to be higher in women than in men ( $p=0.07$ ).The prevalence of endothelin receptor type A gene polymorphisms EDNRA C+1222T was as follows: TT-8.3%, CC- 33.3%, CT-58.6%. The frequency of the genotype CC tended to be higher in women than in men ( $p=0.19$ ).The mean ET-1 level was  $1.49 \pm 0.71$  fmol/ml.The GG genotype of EDNRA C+70G was associated with higher plasma ET-1 concentration in women group ( $p=0.02$ ), moreover the CT genotype of EDNRA C+1222T was related to higher plasma ET-1 concentration in women ( $p=0.006$ ) and in men ( $p=0.02$ ).

**Conclusion** In NTG patients the endothelin receptor type A gene polymorphisms EDNRA (C+70G, C+1222T) may have correlation with higher plasma ET-1 concentration.

• T088

**Intraocular foreign bodies on the Island of Sealand, a preventable serious eye disease**

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**Purpose** To estimate the incidence of ocular injuries with retained intraocular foreign bodies (IOFBs) in the Eastern region of Denmark, and presenting data on aetiology, management, functional and anatomical outcome of these injuries.

**Methods** Search in the national database registry from January 2001 to December 2006 (6 years). Data from matching patient records was recorded. Relevant patients were asked to participate in a late follow-up clinical examination

**Results** 32 patients met inclusion criteria. 69% (22/32) patients participated in a final follow-up examination, and data on the remainder was acquired through relevant patient records at various national clinics. Average follow-up time was 5 years, 4 months. Estimated incidence of IOFBs in Eastern Denmark identified in this study was 0.154 per 100,000. All patients were male. Hammering was the most common mechanism of injury, occurring in 66% of patients. Endophthalmitis was diagnosed in 6% of patients at presentation. Best-corrected visual acuity of the injured eye at final follow-up was 0.5 or better in 47%, 0.5 to 0.1 in 19%, and worse than 0.1 in 34%. Anatomical success, defined as an attached retina and no intravitreal silicon oil, was achieved in 83%.

**Conclusion** The incidence of IOFBs in Denmark seems to be on par with similar recent studies. The demographics are typical for this type of ocular injury. A high rate of anatomical vitreoretinal success was achieved, while a good long term visual function was achieved in half the cases. A considerable proportion of patients sustain severe long-term visual loss in the injured eye despite modern intraocular surgical techniques. Prevention through simple measures remains the mainstay of dealing with this typically work-related and serious eye injury.

**• T089****Choroidal thickness in retinitis pigmentosa using EDI OCT**

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**Purpose** To describe the choroidal thickness of patients with RP using enhanced depth imaging (EDI) and spectral domain (SD) optical coherence tomography (OCT), comparing with impaired non affected subjects.

**Methods** Prospective, case-control study of 20 patients with RP imaged using the Spectralis EDI protocol. 40 healthy age-matched subjects participated in the study as control. RP patients had mild to severe disease, with a visual acuity range of ETDRS 20/20 to light perception. Choroidal thickness measures were determined via manual segmentation of the OCT image. Submacular choroidal thickness measurements were obtained beneath the fovea and at 500 µm intervals for 2.0 mm nasal and temporal to the centre of the fovea. These measurements on RP patients were compared to choroidal thickness measurements from 40 controls with similar refraction and no clinical evidence of retinal or glaucomatous disease. Statistical analysis was performed to compare choroidal thickness at each location between the two groups and to correlate choroidal thickness with best-corrected visual acuity.

**Results** Mean ages were 47.025 years for control patients and 49.3 years for RP patients ( $p>0.05$ ). Mean choroidal thickness measurements were  $211.9 \pm 80 \mu\text{m}$  in RP patients and  $305.6 \pm 89 \mu\text{m}$  in controls ( $p<0.0001$ ). The thickness gradually decreased toward the peripheral retina in the RP and control patients. It was greater in the temporal field than the nasal one in both groups. All values were highly significantly lower in RP group than those of the controls ( $p\leq 0.001$ ). Patients with poorer visual acuity or older (longer duration of disease) tended to have thinner choroids

**Conclusion** Submacular choroidal thickness, as measured by SD-OCT EDI, is significantly reduced in patients comparing with age matched control

**Poster session 2 : Physiology-Biochemistry-Pharmacology / Retina-Vitreous / Neuro-ophthalmology-Strabismology-Paediatric Ophthalmology-History of Ophthalmology**

• F001

**Inflammatory cytokines decrease viability and alter ganglioside profile in retinal pigment epithelium cells**

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**Purpose** Early stages of Age related Macular Degeneration (AMD) are characterized by dysfunction and degeneration of the retinal pigment epithelium (RPE) cells, which participate in the death of the overlying photoreceptors ultimately leading to loss of vision. Gangliosides (GG) make a wide and heterogeneous family of sialic-acid-containing glycosphingolipids, composed of a sugar chain branched on a ceramide. They are major components of cellular membranes, particularly abundant in the brain and nervous tissue, including retina. While their developmental and neuroprotective actions have been demonstrated, their precise role in retina's function and its pathologies is still poorly understood. The present study aimed to investigate the role of GG in the response of RPE cells to inflammation, which is known as one of the pathophysiological features of AMD.

**Methods** Cultured human RPE cells (ARPE19) were exposed to an inflammatory cytokine mixture (ICM): TNF- $\alpha$ , IL-1 $\beta$  and IFN- $\gamma$  for 72 hours. Cell viability was assessed and GG were analyzed by Liquid Chromatography coupled with tandem mass spectrometry.

**Results** ICM had deleterious effects on ARPE19 viability: cell number decreased by half between control and treated conditions. GM3 appeared to be the main GG class present in ARPE19 cells. Interestingly, ICM exposure was associated with modifications in the GM3 profile: relative amounts d18:1/16:0 species increased whereas those of d18:1/24:1 species decreased.

**Conclusion** Our observations suggest that GG might be implicated in ARPE19 cell response to inflammatory cytokines, although the precise biological role of the change in fatty acid profile of GM3 still needs to be clarified.

• F003

**Chicken peptidylarginine deiminase type I and III are constitutively expressed in the retinal neuron**

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**Purpose** Peptidylarginine deiminase (PAD) is a post-translational modification enzyme that catalyzes the conversion of protein-bound arginine residues to citrulline residues in the presence of calcium ion. PAD genes are distributed generally throughout vertebrates. In chickens, three PAD genes have been identified and are orthologous to the mammalian genes encoding PAD1, PAD2 and PAD3, respectively. The expression levels and tissue/cellular location of each PAD under normal physiological conditions have not been elucidated as yet.

**Methods** cPAD1 or cPAD3 specific antibodies were prepared from rabbits immunized with a synthetic peptide and used for western blot analyses and immunohistochemistry assays. To determine the intracellular localization of chicken PADs in retina cells, immunoelectron microscopy was also carried out. Whether or not the deiminated proteins were produced in retinal tissues was also examined by immunohistochemistry using an anti-modified citrulline detection method.

**Results** We found that both cPAD1 and cPAD3 are expressed in the chicken retina. cPAD1 was localized in the inner nuclear layer (INL) whereas cPAD3 was localized in the outer photoreceptor (OP). cPAD3 was present at especially high levels of detection in the lamella structure. Deiminated proteins were also detected in the INL and OP, suggesting that cPAD1 and cPAD3 function in the chicken retina under normal physiological conditions.

**Conclusion** Our findings suggest that chicken PAD1 and PAD3 play a homeostatic role in the chicken retina in governing sight.

• F002

**Blue light toxic action spectrum on A2E-loaded RPE cells in sunlight normalized conditions**

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**Purpose** Sunlight exposure is supposed to induce cumulative damage to the retina in retinal pathologies, such as AMD. The high energy visible spectrum between 380 nm and 500 nm (blue light) is incriminated. The goal of this study was to identify the spectrum of retinal toxicity induced by sunlight in physiological irradiance conditions.

**Methods** RPE cells incubated for 6 hours with 0, 12.5, 20 and 40  $\mu$ M of A2E were exposed for 18 hours to 10 nm illumination bands with the first band centered at 390 nm and going up to 520 nm. Light irradiances were normalized with respect to the natural sunlight reaching the retina after filtering by the eye ocular media. Six hours after light exposure, cell viability, necrosis and caspase-3/7 activity were assessed using the Apoptox-Glo Triplex Assay.

**Results** A2E-loaded RPE cells presented fluorescent bodies within the cytoplasm with a similar spectrum to that of A2E. Exposure to the 10 nm illumination bands induced morphological changes associated to a loss in cell viability. Light toxicity was dependant of A2E concentration and was higher in the blue spectral range with maxima in a specific 30 to 40 nm bandwidth. In addition, caspase-3/7 activity, indicative of cell apoptosis, was highly induced by the same narrow range whereas necrosis was not significantly different to that of cells maintained in darkness.

**Conclusion** We described for the first time the precise spectrum of light toxicity in physiological irradiance conditions on an in vitro model of AMD. The 415-455 nm narrow spectral range generated the greatest phototoxic risk to RPE cells. This provides new information for designing selective protective filters, without disrupting visual and non-visual functions.

• F004

**Does cyclodextrin affect penetration of diclofenac sodium through amniotic membrane?**

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**Purpose** To investigate permeability of amniotic membrane (AM) to diclofenac sodium containing eye drops with or without cyclodextrin (CD)

**Methods** Cryopreserved AM pieces on cellulose acetate filter membranes were mounted in the previously established vertical Franz-diffusion cell system equipped with autosampler. In vitro penetration of two commercially available eyedrops containing 0.1% diclofenac sodium was examined. Voltaren Ophtha CD (VO, with CD) and Unidophen (UN, without CD) were compared. Drug release was determined by quantitative absorbance measurement carried out with a high performance liquid chromatography (HPLC).

**Results** The initial two hours, release of diclofenac from VO was lower than penetration of diclofenac from UN. At 30 minutes only 6.6% of diclofenac penetrated AM from VO vs 11.5% from UN. At 2 hours difference between two eyedrops was not significant (25.69% for VO and 27.39% for UN; p>0.05). After two hours, greater concentration of VO could be measured in the acceptor phase than that of UN and this difference remained significant over the study period. Seven and half hour after instillation we detected 56.26% for VO versus 35.62% for UN of baseline concentrations.

**Conclusion** Drug penetration of diclofenac sodium was affected by CD. Until 120 minutes following instillation, CD decreased drug release from eyedrop containing CD compared to eyedrop without CD. After 2 hours, however drug penetration became significantly greater from solution with CD compared to solution without CD.

**• F005****Assessment of polyesteramide of PEA Microparticles for in vivo intraocular injections**

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**Purpose** To analyze polyesteramide (PEA) microparticles ("MPs") interaction with reagents used in histological analysis and their compatibility with the steps required for ocular injection.

**Methods** Unloaded- and dye-loaded (Chromoionophore II) PEA III MPs suspended in PBS/mannitol were: i) analyzed under light and fluorescence optic microscopy; ii) exposed to reagents, solutions and processes required to perform histological procedures; iii) released through different needle gauges and quantified in a Neubauer chamber. Rat eyes were divided into two groups: intravitreal injection (n= 20) and subtenon/subconjunctival injection (n=24). After sacrifice, tissues were processed as retinal whole-mount and cryosections.

**Results** i) PEA III MPs were stable when contacted with distilled water, PBS, 4% PF and 2% glutaraldehyde; ii) PEA III MPs lost shape or dissolved when in contact with Xylene, EtOH or acetone; iii) freezing at -20°C and heating at 60°C does not impact particles re-dispersion, shape and polydispersity; iv) no significant differences in MPs number released through different needle gauges (25G, 27G, 30G, 32G) were found; v) in unstained tissues, MPs preserved their morphological characteristics and coalesced into depots.

**Conclusion** MPs: i) are compatible with some of the steps required for routine histological processing. ii) can be released through needle gauges used in human clinical setting; iii) the injection should be done during the 30 minutes following suspension preparation.

**• F007****Aquaporin expression in the human retinal pigmented epithelial cell line ARPE-19**

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**Purpose** Aquaporins (AQPs) are a family of transmembrane channel proteins permeable to water. They are likely to play an essential role in the outer blood retinal barrier allowing passive water transport through the retinal pigmented epithelium (RPE). The identification of AQPs expressed in the RPE could contribute to a better understanding of the development of macular edema in several eye diseases such as diabetic retinopathy. However, the type of AQPs expressed by the RPE is currently a subject of controversy.

**Methods** We investigated the expression of AQPs in the human RPE cell line ARPE-19, a model of human RPE. First, AQPs mRNA expression was determined by quantitative real-time PCR (qRT-PCR). Furthermore, protein expression was analyzed by both immunofluorescence (IF) and Western blot (WB).

**Results** The mRNA of AQP1, AQP4, AQP6 and AQP11 were expressed in ARPE-19 cells. However, the expression of AQP3, AQP4, AQP6, AQP7, AQP8, AQP9, AQP11 and AQP12 were found by IF. Finally, the expression of AQP4 and AQP6 was found by WB.

**Conclusion** In conclusion, several AQPs were detected both at the mRNA and at the protein level in ARPE-19 cells and may account for water movement in those cells.

**• F006****A drug-induced central retinal vein occlusion**

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**Purpose** To report a case of central retinal vein occlusion probably caused by tranexamic acid medication.

**Methods** A 45-year-old woman consulted in our emergency room because of a visual field defect in her left eye without visual loss. Her past medical history revealed metrorrhagias treated by tranexamic acid and no cardiovascular disease. Visual acuity was 20/20 in both eyes. Slit-lamp examination was normal in both eyes and fundus examination showed peri-papillary haemorrhages LE. Two days later she complained of sudden visual loss LE. Visual acuity of her left eye was limited to light perception. Fundus examination revealed many haemorrhages, macular edema, and papillary edema. Fluorescein angiography showed central retinal vein occlusion associated with a cilioretinal occlusion. General and biological examinations revealed no abnormalities. Systemic disorders were excluded. Therefore we concluded to a iatrogenic pathology.

**Results** In our patient after excluding systemic disorders potentially responsible for this ocular pathology we suspected a iatrogenic cause. Tranexamic acid is a classical treatment for haemorrhages especially in case of metrorrhagias. It allows haemostasis thanks to an antifibrinolytic action due to plasminogen inhibition. A few cases of vein and arterial occlusions caused by tranexamic acid treatment have been reported in literature.

**Conclusion** In a young patient without systemic disorder presenting with a retinal vascular occlusion we must always keep in mind a drug-induced pathology.

**• F008****Serum concentrations of infliximab in patients with Behcet's disease**

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**Purpose** We investigated the relationship between the serum concentrations of infliximab (IFX), an anti-tumor necrosis factor-alpha monoclonal antibody, and its therapeutic effect in patients with Behcet's disease (BD) who were receiving IFX therapy.

**Methods** Nine BD patients (7 males and 2 females) with uveitis who were receiving IFX therapy for 1 years or longer were included in this study. IFX (5-6 mg/kg) was administered at weeks 0, 2, and 6 and thereafter every 8 weeks. After informed consent was obtained, peripheral blood samples were collected before the IFX infusion. The serum concentrations of IFX were measured by enzyme-linked immunosorbent assay.

**Results** In the 6 patients who did not develop any uveitis attacks, the mean serum concentration of IFX was 3.6-13.5 µg/ml, while the mean concentration was < 0.1-1.58 µg/ml in three patients who had uveitis attacks. We speculate that the uveitis attacks occurred in the three patients because of the low IFX concentrations, and shortened the IFX administration interval from 8 weeks to 6 or 7 weeks. Thereafter, two of the three patients did not have any uveitis attacks. The serum concentration of IFX in the two patients was increased from 0.1 to 5.5 µg/ml and 0.2 to 3.6 µg/ml, respectively, whereas the concentration in one patient who had uveitis attacks was < 0.1 µg/ml, and the neutralizing antibody titre to IFX was negative. The serum concentration of IFX was correlated with its efficacy.

**Conclusion** Measurement of IFX serum concentration is useful to predict treatment efficacy in BD patients.

**Poster session 2 : Physiology-Biochemistry-Pharmacology / Retina-Vitreous / Neuro-ophthalmology-Strabismology-Paediatric Ophthalmology-History of Ophthalmology**

• F009

**Suppression of TRPV 1 channels activity is a possible way in treatment of ophthalmic diseases**

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**Purpose** The superfamily TRP (Transient Receptor Potential Channels), including subfamily TRPV (1-6) channels, play an important role in regulation of eye homeostasis and development of ophthalmic pathology (Zhang F. et.al., 2007). The purpose of the paper is to study the possibility of suppression of TRPV1 channels activity that can promote braking of pathological processes in the cornea and the retina.

**Methods** In experiment the action of laminaria dry extract is investigated on the stable line of CHO cells, expressing the receptor of the rat TRPV1 (rTRPV1). This line was received with use of the T-Rex System (Invitrogen). cDNA coding rTRPV1, was cloned in an induced expressed vector of pcDNA4/TO. The expression of TRPV1 was induced by addition of tetracycline on cellular environment (1mg/ml) in 24 hours prior to testing. The selected monoclonal antibodies with the greatest level of an expression of TRPV1 were used. The fluorescent analysis was carried out with the automatic system of dispensing of NOVOSTar liquids (BMG LABTECH). Change of cellular fluorescence ( $\lambda_{\text{ex}}=485 \text{ nm}$ ,  $\lambda_{\text{em}}=520 \text{ nm}$ ) before and after stimulation of cells agonist TRPV1 - capsaicin (400 nM) in the presence of various concentrations of laminariadry extract was studied. Measurements were performed at pH7.4 and 37 °C.

**Results** In experiment it was established that an application of the laminaria in concentration of 4-5 mg/ml on cells with an expressed receptor of TRPV1 considerable falling (30-50%) Ca<sup>2+</sup> response of cells at a single activation of cells with the capsaicin.

**Conclusion** Thus, the extract of laminaria shows a considerable inhibiting activity in relation to TRPV1 channels. It may be one of the ways in the treatment of ophthalmic pathology.

• F010

**Effect of systemic hyperoxia on retinal oxygen saturation**

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**Purpose** Several groups have reported that 100% oxygen breathing caused an increase in retinal venous oxygen saturation. In the present study we set out to study the effects of 100% oxygen breathing on retinal blood flow and retinal oxygen saturation to gain more insight into the regulation of retinal oxygenation.

**Methods** 30 healthy volunteers were included into the present study. The effect of 100% oxygen breathing on retinal blood velocities was performed using laser Doppler velocimetry (LDV). Retinal vessel diameters were measured with the Dynamic Vessel Analyzer (DVA, Imedos, Germany). Retinal oxygen saturation was evaluated in retinal arteries and retinal veins using spectroscopy also employing the DVA. The effect of 100% oxygen breathing on systemic oxygen tension was evaluated based on arterialized blood samples drawn from the earlobe.

**Results** Breathing 100% oxygen caused systemic hyperoxia as evidenced from a pronounced increase in systemic oxygen tension ( $p < 0.001$ ). Oxygen saturation increased in both retinal arteries and retinal veins ( $p < 0.05$  each) but the effect in veins was more pronounced. As expected we observed a decrease in retinal vessel diameters, retinal blood velocities as well as in retinal blood flow ( $p < 0.001$  each).

**Conclusion** The results are compatible with the idea that increased venous oxygen saturation during 100% oxygen breathing is due to a pronounced decrease in retinal blood flow. In addition, our data indicate unchanged retinal oxygen extraction during hyperoxia.

• F011

**Reproducibility of retrobulbar blood flow velocity measurements using two different colour Doppler imaging devices**

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**Purpose** To evaluate the intra and inter-operator reproducibility of color Doppler imaging (CDI) in assessing blood flow velocity in the ophthalmic (OA), central retinal (CRA) and short posterior ciliary arteries (SPCA)

**Methods** The right eye of two group of 8 healthy volunteers was examined. Two radiologists and two ophthalmologists, divided into pairs, measured Peak Systolic Velocity (PSV), End-Diastolic Velocity (EDV) and Resistivity Index (RI) of each vessel using a different CDI device for each group. The concordance between two measurements was evaluated with Lin's concordance correlation coefficient (CCC)

**Results** Globally very good degrees of intra-operator concordance were obtained for the PSV (0.859), EDV (0.834) and RI (0.859) of the OA. There was moderate concordance for PSV (0.574) and EDV (0.594) and good concordance for RI (0.694) for the CRA. Good degrees of concordance were obtained for the SPCA measurements. However inter-operator concordance was found globally poor

**Conclusion** These data show that retrobulbar vessels CDI measurements are operator dependent. To increase the intra-operator and inter-operator concordance rules should be adopted for timing of the exam and probe position to minimize the pressure applied on the eye

• F012

**Angiogenic role of glycerol in laser-induced choroidal neovascularization**

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**Purpose** It has been reported that intravitreous administration of anti-mouse IL18 antibody increases laser-induced choroidal neovascularization (CNV) in mice (Doyle et al. Nat Med 2012). However, since those experiments compared an anti-mouse IL18 antibody to a sham injection group, we tested the putative angiogenic effects of this antibody using biologically and chemically rigorous controls in order to clarify the role IL18 in laser-induced CNV.

**Methods** Laser photocoagulations were performed to perforate Bruch's membrane followed by intravitreous injections, and the eyes were collected and analyzed on day 7 after laser treatment. Laser-induced CNV lesions were stained with isolectin B4 and volumes quantified using z-stack on a confocal microscope system (Leica). We tested the Abcam anti-mouse IL18 antibody used by Doyle et al. which was dissolved in 50% glycerol, an MBL anti-mouse IL18 antibody, as well as isotype control antibodies in appropriate control buffers.

**Results** We found that MBL anti-mouse IL18 antibody neutralized IL18-induced cellular signaling whereas the Abcam antibody did not do so. We also found that the Abcam IL18 antibody increased CNV whereas the MBL antibody did not. We found that glycerol increased CNV to the same extent as the Abcam IL18 antibody, and that the Abcam IL18 antibody in glycerol did not increase CNV in mice deficient in Aquaporin-1, a channel that is permeant to glycerol and promotes angiogenesis.

**Conclusion** IL18 blockade does not increase CNV. The previously reported results are an artifact of pro-angiogenic glycerol in which the Abcam IL18 antibody is dissolved.

**Poster session 2 : Physiology-Biochemistry-Pharmacology / Retina-Vitreous / Neuro-ophthalmology-Strabismology-Paediatric Ophthalmology-History of Ophthalmology**

• F013

**IL18 does not reduce choroidal neovascularization and causes retinal dysfunction in mice**

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**Purpose** It was reported that intravitreous administration anti-mouse IL18 antibody increases the size of laser-induced choroidal neovascularization (CNV) lesions in mice (Doyle et al. Nat Med 2012). IL-18 administration was therefore proposed as a potential therapeutic for CNV, although this hypothesis was never directly tested. Here, we determined the effect of IL-18 injection on laser CNV in mice, and also show for the first time that IL-18 is toxic to retinal function.

**Methods** Laser photocoagulations were performed to perforate Bruch's membrane followed by intravitreous injections of recombinant IL-18 (rIL-18) or PBS, and the eyes were collected and analyzed on the day 7 after laser treatment. Laser-induced CNV lesions stained with isolectin B4 were imaged by a confocal microscope system (Leica). Mice receiving rIL-18 or PBS were subjected to electroretinography 7 days after sub-retinal injection. RPE flat mounts of rIL-18 or PBS treated wild type, Fas<sup>-/-</sup>, and FasL<sup>-/-</sup> mice were stained for intercellular junctions.

**Results** Average CNV volume was not different in eyes treated with recombinant IL-18 compared to vehicle treated. Wild-type mice receiving rIL-18 had RPE degeneration, but the degeneration did not occur in Fas or FasL knockout mice. We also injected plasmids coding pro-IL-18 and mature IL-18, and found mature IL-18 didn't change CNV volume but caused degeneration. Wild type mice receiving rIL-18 injections showed lower amplitude in electroretinography compared to vehicle injected eyes.

**Conclusion** IL-18 injections did not reduce CNV. Also, IL-18 administration induced RPE degeneration via a Fas/Fas ligand-dependent pathway.

• F014

**Circulating blood glutathione levels influence retinal microvascular function in healthy individuals**

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**Purpose** To assess the relationship between retinal vessel reactivity and oxidative stress markers in the systemic circulation.

**Methods** Two hundred and twenty four healthy volunteers (aged 20-70 years) free of cardiovascular risk factors based on medical history, lipid profiles, blood pressure, and body mass index were included in this study. Measurements included plasma cholesterol, blood glucose, triglycerides, oxidized (GSSG) and reduced (GSH) forms of glutathione. Arterial and venous retinal vessel reactivity was assessed using the dynamic retinal vessel analyser (DVA, IMEDOS, Germany) according to an already established protocol.

**Results** A simple correlation model showed that arterial and venous dilation amplitude was inversely with correlated with age ( $r = -0.4041$ ,  $p = 0.0001$ ;  $r = -0.4045$ ,  $p = 0.0001$ , respectively). Forward step-wise multiple linear regression analysis revealed that independent of age and other systemic influences, blood GSH levels were positively correlated with arterial and venous percent dilation ( $\beta = 0.158$ ,  $p = 0.0133$ ;  $\beta = 0.224$ ,  $p = 0.0004$ , respectively), and overall dilation amplitude ( $\beta = 0.245$ ,  $p = 0.0003$ ;  $\beta = 0.230$ ,  $p = 0.0006$ , respectively). In addition, blood GSH levels negatively influenced the arterial constriction response slope (SlopeAC:  $\beta = -1.722$ ,  $p = 0.0024$ ), while GSH and overall redox status (GSH/GSSG) influenced percent constriction in both the artery ( $\beta = -0.345$ ,  $p = 0.0002$ ,  $\beta = -0.229$ ,  $p = 0.0462$ , respectively) and vein ( $\beta = -0.253$ ,  $p = 0.0001$ ;  $\beta = -0.156$ ,  $p = 0.0177$ , respectively).

**Conclusion** In otherwise healthy individuals, retinal microvascular function is influenced by systemic anti-oxidative capacity.

• F015

**RESVEGA, by the presence of Resveratrol, inhibits retinal endothelial tube formation**

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**Purpose** Resveratrol (RES) is a polyphenol with various properties. RESVEGA, a food supplement developed by Thea, contains 2% of RES. The aim of this study was to examine the activity of RES and RESVEGA on endothelial cells.

**Methods** Compounds activity was tested using an in vitro angiogenesis assay. Endothelial cells obtained from human umbilical vein (HUVEC) and monkey retinal endothelial cells (RF/6A) were used for this assay. 96-well cell cultures plates were coated with Matrigel. Cells (15000 per well) were incubated in the presence of RES, RESVEGA or vehicle. Tube formation was recorded every 15 min during 5 hours using Time Lapse Video Microscopy. At each time point, 3 fields per well were photographed and images were analysed using Metamorph software. Each experiment was repeated three times, with triplicate samples.

**Results** RES and RESVEGA products activities were compared at the following concentrations: 25, 50 and 100  $\mu$ M. Both RES, tested on HUVEC and RF/6A, and RESVEGA, tested on RF/6A, reduced the ability of endothelial cells to form vascular networks on Matrigel. This inhibitory effect was dose dependent in the range of 25-100  $\mu$ M.

**Conclusion** This is the first report of the anti-angiogenic effect of RES on RF/6A retinal endothelial cells. RESVEGA supplement presents the same anti-angiogenic activity than resveratrol alone.

**Commercial interest**

• F016

**Correlation between SD-OCT, immunocytochemistry and functional findings in an animal model of retinal degeneration**

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**Purpose** The P23H rhodopsin mutation is an extensively studied model of ADRP. We evaluated the anatomical changes using SD-OCT and correlate the findings and retinal thickness values with immunocytochemistry. Functional changes were analyzed.

**Methods** Heterozygous P23H pigmented transgenic rats aged from P18 to P180 were studied. LE rats bred with Sprague Dawley (SD) 1 month old served as wild type controls. Visual acuity and contrast sensitivity evaluation was performed every month. Corneal ERGs were recorded under scotopic and photopic conditions. Retinal thicknesses at different levels (total thickness, ONL + RPE, ONL and IPL), fundus autofluorescence (FAF) and fluorescein angiography was performed in 3 animals at P150 using Spectralis OCT and HRA (Heidelberg Engineering, Germany). Retinas were immunostained for ICC.

**Results** Retinal thickness diminution was seen in OCT sections, with a clear loss of ONL and morphological modifications. Statistically differences were found between groups in all evaluated thicknesses. In the P23H rats, change in FAF was noted comparing to control group, as sparse autofluorescent dots. No relevant changes were observed in the angiography pattern. ICC showed a progressive decrease in ONL thickness. Functional changes were progressive with time.

**Conclusion** Anatomical changes in pigmented P23H can be observed using SD-OCT and immunocytochemistry, with a good correlation between their values. SD-OCT and FAF are important tools for research in retinal degenerations.

**Poster session 2 : Physiology-Biochemistry-Pharmacology / Retina-Vitreous / Neuro-ophthalmology-Strabismology-Paediatric Ophthalmology-History of Ophthalmology**

• F017

**Fundus autofluorescence, OCT thickness evaluation, angiography and immunohistochemistry correlation in albino P23H rats**

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**Purpose** To evaluate the correlation between OCT changes and fundus autofluorescence (FAF) with immunohistochemistry (ICC) findings in an animal model of RP, the P23H rat and to investigate retinal and choroidal vascularization using fluorescein and indocianin green angiography

**Methods** Twenty albino homozygous P23H line 1 rats aging from P18 to 27 months and wild-type albino Sprague-Dawley (SD rats) (2 and 11 months old) were used for this study. Normal pigmented Long Evans (LE) 2 months old were used to compare FAF findings. SLO imaging and OCT were acquired using an Spectralis system (Heidelberg Engineering, Germany). For checking FAF, fluorescence was excited using diode laser at 488 nm. Thickness measurements were evaluated avoiding the ones close to the optic nerve. ICC was performed to correlate with the findings of OCT and AF changes

**Results** During the course of P23H degeneration, the FAF pattern varied from not findings in young animals, some spotting at 2 months old to a mosaic of hyperfluorescent dots in the rats of 6 months or older. Retinal thicknesses diminished during the time. P23H rats showed great changes in morphology in advanced ages. Mean retinal thickness values varied from 189.88 µm at P60 to 58.15 µm at 27 months old. Retinal vascular plexus were diminished with time, and vessels exhibiting an abnormal, tortuous morphology could be observed.

**Conclusion** FAF is a non-invasive procedure that can detect changes in metabolic activity at the RPE in animal models of retinal degeneration *in vivo*. OCT and ICC show a good correlation. Retinal vascular plexus changes with aging.

• F019

**Proteomic analysis of the retina in mice deficient in glial fibrillary acid protein and vimentin**

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**Purpose** Investigate the mechanisms underlying functional differences observed in glial fibrillary acid protein (GFAP) and vimentin knockout mice.

**Methods** Retinal tissue was harvested from 21-day-old mice lacking GFAP and vimentin (GFAP(-/-)vim(-/-); n=6), and age-matched wild-type (wt; n=6) controls. The retinal samples were evaluated by two-dimensional polyacrylamide gel electrophoresis, SYPRO Ruby fluorescent stain, and PDQuest software image analysis. Protein spots that were differentially expressed between the two groups were processed for identification by tandem mass spectrometry, prior to further ongoing investigations with western blotting and immunocytochemistry.

**Results** Thirteen protein spots were found to be differentially expressed between the GFAP(-/-)vim(-/-) and wt mouse retinas, as determined by Mann Whitney U test with a significance level at p<0.05.

**Conclusion** The proteins identified are involved in a wide range of biological processes that include glial cell differentiation and development, neuroprotective and neurotrophic actions, redox homeostasis, cell adhesion and migration, and cytoskeletal remodeling. This study will further our understanding of the role of GFAP and vimentin in the retina, and likely provide new insights into various conditions that involve retinal cell degeneration and glial cell activation.

• F018

**Ocular and systemic distribution of MRZ-99030 in pigmented mouse, rat, rabbit and non-human primate by quantitative tissue analysis**

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**Purpose** The ocular tissue distribution and systemic pharmacokinetics of MRZ-99030 was determined after single or repeated topical ocular dose regimens of MRZ-99030 to pigmented mice, rats, rabbits, and non-human primates. MRZ-99030, an amyloid- $\beta$  aggregation modulator, is currently under development for the treatment of glaucoma and AMD, which are the leading causes of blindness and represent diseases with high unmet medical need.

**Methods** Dutch Belted rabbits, Cynomolgus monkeys, Dark Agouti rats, and C57/BL6 mice received single or repeated bilateral topical ocular doses of MRZ-99030. Ocular tissues and plasma were collected up to 24h after dosing and analyzed for MRZ-99030 by liquid chromatography-mass spectrometry (LC-MS).

**Results** MRZ-99030 was readily absorbed into ocular tissues for all species with highest concentrations observed in the cornea and conjunctiva. MRZ-99030 was also found in the ocular tissues of the posterior segment (choroid with RPE and retina) at all time points. Pharmacokinetics show MRZ-99030 in all tissues and systemically, with elimination half-lives that indicate somewhat sustained concentrations in melanin containing tissues. Concentrations in ocular tissues, in particular for the retina, increase with an increase in dose level (mouse and rat) and dose frequency (all species).

**Conclusion** The results show that MRZ-99030 is absorbed into the ocular tissues in all species following topical administration and is readily distributed to the posterior segment of the eye, including the retina. Therefore, this finding strongly supports that MRZ-99030 is able to modulate the A $\beta$  aggregation in the target tissue of glaucoma and AMD.

• F020

**The frequency of idiopathic retinal detachment has seasonal variation and correlated with climate – A survey in Japan**

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**Purpose** Several studies have reported that rhegmatogenous retinal detachment (RRD) has a high incidence in the summer season and it correlates with increased sunshine hours and the average temperature. However, there is no report in Japan where seasonal changes in climate are rather distinct. In this study, we examined the seasonal variation of the frequency of RRD and the influence of climate. Asahi General Hospital is located in Asahi city (Chiba, Japan) where there is only a small change of population and furthermore, there is no other hub hospital in the city.

**Methods** Medical records of consecutive 271 patients (>13-year-old) who had surgery for idiopathic RRD at Asahi General Hospital from January 2007 to December 2011 were retrospectively reviewed. The incidence of RRD was counted by months and seasons, and its seasonal variation and the correlation with the climate factors including the sunshine hours, temperature, humidity, and rainfall were examined.

**Results** 271 RRD eyes of 271 patients (176 male and 95 female, 56.0±15.0 years old) were included in the study. The RRD occurred most frequently in early summer (May to June, 21%), and least frequently in winter (January to February, 14%). Its frequency was significantly correlated with increased sunshine hours ( $r=0.71$ ,  $P<0.05$ ).

**Conclusion** The incidence of RRD has an increasing trend in summer season in Japan, similarly to the previous reports in Asia, Middle East and Europe. It also positively correlated with increased sunshine hours and average temperature. These findings could suggest the influence of increased opportunity of outdoor-activities and miosis by sunshine on the occurrence of RRD

**Poster session 2 : Physiology-Biochemistry-Pharmacology / Retina-Vitreous / Neuro-ophthalmology-Strabismology-Paediatric Ophthalmology-History of Ophthalmology**

• F021

**Outcomes of vitrectomy for vitreous haemorrhage (VH) in patients with presumed choroidal neovascularization (CNV)**

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**Purpose** To evaluate the role of vitrectomy for eyes with dense VH presumed secondary to CNV, as well as their clinical outcomes.

**Methods** Retrospective, consecutive case series from a single centre of 11 eyes of 11 patients over 2 years who underwent vitrectomy, and with no other identified ocular pathology e.g. diabetic retinopathy or trauma.

**Results** Visual acuity (VA) improved in 9 (82%) patients with mean logMAR VA change of 1.19 (+/- 0.69). Prior to vitrectomy, intravitreal anti-vascular endothelial growth factor (anti-VEGF) had been given to 2 patients; pneumatic displacement of submacular haemorrhage to 1 patient; and photodynamic therapy with verteporfin to 1 patient. There were no pre-existing retinal breaks found in all patients intraoperatively. Intraoperative complications include 1 posterior capsule rupture with anterior chamber intraocular lens insertion and 2 iatrogenic retinal breaks treated with laser. Angiography identified polypoidal choroidal vasculopathy in six, occult CNV in one and retinal pigment epithelium rip but no identifiable vascular lesion in one. Angiography was not done in three due to extensive disciform scarring. Lesions beyond the vascular arcade were found to have better prognosis. Recurrence of vitreous haemorrhage occurred in 2 patients.

**Conclusion** Vitrectomy for patients with dense VH and presumed CNV (most commonly PCV) was found to be useful to improve visual acuity, especially for lesions beyond the vascular arcade, although effect is limited. Vitrectomy also allows for subsequent retinal evaluation and angiography. The absence of pre-existing retinal tears is in agreement with the reported outcome that the pathophysiology of breakthrough VH does not involve retinal tears.

• F022

**Simultaneous SD-OCT and fundus autofluorescence imaging of the macula after successful repair of rhegmatogenous retinal detachment**

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**Purpose** To evaluate the ultrastructural changes in the macula after successful repair of rhegmatogenous retinal detachment (RRD) using spectral domain optical coherence tomography (sdOCT) and fundus autofluorescence (FAF) imaging

**Methods** Simultaneous sdOCT (Topcon 2000 FA plus) and FAF imaging were performed with a system for FAF imaging using a fundus camera that included Spaide Autofluorescence filters available on IMAGEnet.

**Results** 31 patients with macular-off RRD and 2 with macular-on RRD were recruited. Post operative visual acuity ranged from 20/400 to 20/20. SdOCT images were normal in 18 eyes (54.5%), revealed macular edema in 9 eyes (27.2%), macular hole in 2 eyes (6%), macular pucker in 3 eyes (9%), submacular fluid in 2 eyes (6%). Fundus autofluorescence imaging of the macula were normal in 13 eyes (39.3%). There were central abnormalities in 14 eyes. Patients with abnormal FAF were also associated with poor postoperative BCVA ( $p<0.001$ )

**Conclusion** The present study demonstrates the ultrastructural changes assessed with simultaneous FAF and sdOCT and their relation with visual outcome

• F023

**Cytologic analysis in vitreo-retinal surgery**

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**Purpose** Proliferative vitreoretinopathy (PVR) is the leading cause of failure in retinal detachment surgery. The migration of retinal pigment epithelial cells and the proliferation of the extracellular matrix has been associated with PVR. The purpose of this prospective study was to compare the cellular contents of the vitreous of patients undergoing retinal detachment surgery with patients scheduled for macular surgery.

**Methods** The vitreous samples of patients with epiretinal membrane and rhegmatogenous retinal detachment were obtained at the initial phase of surgery without previous intraocular infusion. A cytological analysis and Papanicolaou staining was performed in all cases. The concentration of vitreous cells in 62 patients with retinal detachment and 62 undergoing macular surgery (52 epiretinal membranes, 6 macular holes and 4 vitreomacular traction syndromes) were analyzed semi-quantitatively.

**Results** An increased concentration of vitreous cells and free pigment was observed in a higher proportion in retinal detachment specimens compared with patients undergoing macular surgery. In the retinal detachment group, there was a higher amount of hyalocytes ( $p=0.017$ ) and macrophages ( $p=0.00865$ ). There was no correlation between any cell concentration and PVR induced recurrence of retinal detachment. Interestingly, in the vitreous of eyes with vitreomacular traction, no cells could be detected.

**Conclusion** Vitreous changes observed in patients with retinal detachment appear to involve vitreous cells particularly hyalocytes and macrophages, whereas in vitreomacular traction, syndromes no cellular reaction could be detected.

• F024

**Long-term results and prognostic factors for visual acuity after diabetic vitrectomy: A 10-year follow-up study**

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**Purpose** To report long-term results and estimate long-term prognostic factors for visual acuity following modern diabetic vitrectomy.

**Methods** Retrospective review of patient files from a large diabetes center between 1996 and 2010. Surgical history was obtained from the Danish National Patient Register. Follow-up intervals were 3 months and 1, 3, 5 and 10 years after surgery.

**Results** In total, 167 patients had diabetic vitrectomy. Surgery indications were non-clearing vitreous hemorrhage (47%) or tractional retinal detachment (53%). Median visual acuity increased from 0.06 before surgery to 0.21 after surgery ( $p<0.001$ ) and stabilized. The proportion of patients with reading ability stabilized as from the 3-month follow-up. After 10 years, 66% of the operated eyes were pseudophakic or aphakic and 13% carried silicone oil. Preoperative iris rubeosis was correlated with inability to read after 1 and 3 years ( $p<0.04$ ). Use of silicone oil was correlated with inability to read at all follow-up examinations ( $p<0.05$ ).

**Conclusion** After diabetic vitrectomy, visual acuity improved and stabilized as from the 3-month follow-up. The most consistent long-term predictor of unfavorable visual acuity was use of silicone oil as tamponade agent. The results can be used to counsel patients on the risk of complications after surgery.

**Poster session 2 : Physiology-Biochemistry-Pharmacology / Retina-Vitreous / Neuro-ophthalmology-Strabismology-Paediatric Ophthalmology-History of Ophthalmology**

• F025

**Idiopathic macular hole closure toward the optic disc after internal limiting membrane peeling**

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**Purpose** To compare the distance between the optic disc and the fovea before and after successful closure of a macular hole (MH) by pars plana vitrectomy (PPV) with internal limiting membrane (ILM) peeling. In addition, to investigate whether the distance is significantly correlated with the retinal thickness around the MH.

**Methods** Thirty-seven eyes of 36 patients with an idiopathic MH that underwent PPV with ILM peeling were studied. The distance between the point of intersection of the disc margin with a temporal-inferior vessel and the center of the opened or closed MH was measured manually with the Spectralis HRA+OCT (Heidelberg Engineering, Germany). The retinal thickness of macula was also measured by the volume scan mode.

**Results** The mean disc-foveal distance was significantly shorter postoperatively than preoperatively ( $3783.7 \pm 308.5$  um and  $3914.5 \pm 320.4$  um, respectively;  $P<0.0001$ ). The distance in eyes with stage 3 and 4 MH ( $n=22$ ) was significantly greater than in eyes with stage 2 MH ( $n=15$ ) ( $136.7 \pm 134.9$  um vs.  $107.8 \pm 107.7$  um, respectively;  $P=0.012$ ). The distance in stage 3 and 4 MHs ( $n=21$ ) was weakly but significantly correlated with the mean postoperative parafoveal nasal thickness ( $r=0.45$ ;  $P=0.038$ ).

**Conclusion** The significantly shorter postoperative disc to fovea distance in eyes with closed MH especially in stage 3 or 4 MH suggests that the MH moves nasally after PPV with ILM peeling.

• F026 / 2417

**Intravitreal drug dispersion and needle gauge**

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**Purpose** Intravitreal injections (IV) have become the main treatment for many ocular diseases. At present, IV are supplied with a 27G, 30G needles. Reduced ocular discomfort and scleral penetration forces have been reported after using smaller gauge needles. However, smaller inner diameter implies increased fluid speed and intraocular turbulence that may result in retinal harm.

**Methods** IV were injected with 0.05 mL Indian ink using 27, 30 and 32 G needles. After the injection the eyes were divided in groups and the vitreous was photographed. Three masked observers scored Indian ink dispersion within the vitreous gel from 1 (completely localized) to 4 (complete dispersion).

**Results** Intra-observer and inter-observer reproducibility was between 0.78 and 0.95 and between 0.93 and 0.95, respectively (intra-class correlation coefficient). The average Indian ink dispersion scores for 27, 30 and 32 G needles were 3.4, 2.5 and 1.8 respectively ( $p=0.07$  for 27 vs. 32 G;  $p=0.71$  for 27G vs. 30G;  $p=0.15$  for 30G vs. 32G; Student t test for unpaired data)

**Conclusion** The inner flow is four times faster in thinner needles, considering that the plunger slides with similar speed. We might expect that flow through the thinner needle would induce more turbulence within the vitreous gel; however, the pattern of intravitreal distribution of Indian ink suggests that the injected fluid and the turbulence were confined to a small bag in the area where smaller gauges were used. The results of this work suggest that 32G needles do not increase turbulence of injected fluid. Limited turbulence may be of even greater interest in elderly eyes with liquefied vitreous gel, reducing mechanical retinal damage

• F027 / 2416

**Baseline characteristics and vitreoretinal interface features in patients with vitreomacular traction without macular hole from the MIVI-TRUST clinical program**

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**Purpose** There is limited published evidence on demographic and vitreoretinal interface (VRI) characteristics of patients with persistent vitreomacular traction (VMT). Establishing characteristics of untreated VMT may contribute to a better understanding of the burden of VMT disease. To describe baseline characteristics and VRI features in patients with persistent VMT included in the phase 3 ocriplasmin studies. This analysis reports on VMT patients without macular hole (MH)

**Methods** Two randomized, double-masked, placebo-controlled trials designed to determine efficacy and safety of ocriplasmin for the treatment of VMT comprising 652 patients. Key baseline characteristics included patient demographics (age, gender); eye-disorder (time since diagnosis, visual acuity (VA) study eye (SE), non-study eye (NSE), presence of pseudophakia or ERM); VRI features (type of focal VMA, vitreous separation); VFQ-25 composite score

**Results** VMT without MH was present in 499 patients (76.5%). Baseline characteristics were: 72.6 years (SD 9.8); 62.7% female. Time since diagnosis was: 268 days (SD 547); VA: SE 66.8 (SD 11.2); NSE 73.5 (SD 16.5). Pseudophakes: 38.7%, concomitant ERM: 46.3 %. The majority of patients presented with focal adhesion  $\leq 1500$  microns (69%) and with vitreous adhesions attached at fovea and optic nerve (92%); VFQ-25 composite score: 78.0 (SD 15.2)

**Conclusion** Baseline characteristics and vitreoretinal Interface features in patients with vitreomacular traction without macular hole from the MIVI-TRUST Clinical Program

• F028

**Late results of sclera reconstructive surgery aimed at preventing the progression of myopic macular dystrophies**

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**Purpose** To assess the long-term efficiency of scleroplasty performed using a simplified technique of buckling the posterior pole in patients with congenital myopia complicated by macular dystrophy and posterior staphyloma of the sclera

**Methods** The surgery was performed according to Snyder-Thompson technique in which the buckle made of a biologically active synthetic graft was sutured using a simplified technique of its localization at the posterior pole. 42 surgeries were administered to 34 patients aged 13–43 with myopia of  $-11.5$  to  $-31.0$  D. The shape and stage of staphyloma was determined by ultrasound scanning or with an ophthalmoscope. The follow-up period reached 3–8 yrs ( $4.2 \pm 1.3$  yrs).

**Results** Next day after surgery, flattening of the staphyloma can be seen on B-scan. The axial length becomes 1.0–2.0 mm shorter, the acoustic density of the posterior pole of the sclera increases by 5.3 dB, visual acuity shows a 0.1–0.3 growth, while the refraction is 1.5–2.5 D lower. The late follow-up results (6–8 years) demonstrated myopia stabilization in 90.2% of cases. Myopia progression rate turned out to be half as slow as prior to the surgery, and B-scan showed a sustainable flattening of the staphyloma. Over the whole observation period, no new pathological processes emerged in the macular area, while no existing pathological processes showed progression

**Conclusion** Scleroplastic surgery combined with posterior pole buckling with a biologically active synthetic graft should be recommended for the treatment of high complicated myopia, the prevention of staphyloma progression, myopic macular dystrophies and vitreomacular traction syndrome.

**Poster session 2 : Physiology-Biochemistry-Pharmacology / Retina-Vitreous / Neuro-ophthalmology-Strabismology-Paediatric Ophthalmology-History of Ophthalmology**

• F029

**Live retinal image mosaicking during fundus examination with a computer-assisted slit-lamp prototype**

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**Purpose** Laser photocoagulation is the standard treatment for sight-threatening diseases worldwide, namely diabetic retinopathy. The slit lamp biomicroscope coupled to the laser permit the visualisation of only a small portion of the retina, complicating the task of localizing and identifying laser targets, increasing treatment duration and patient discomfort. Aim: to assist ophthalmologist, we propose a method for creating intra-operative retina maps for view expansion using a slit-lamp biomicroscope

**Methods** Based on the mosaicking method previously described by Richa R (Medical Image Computing and Computer-Assisted Intervention (MICCAI'12) the method was a combination of direct and feature-based methods, suitable for the textured nature of the human retina. We used two major enhancements to the original formulation: (1) an efficient pixel selection scheme for increased computational efficiency; (2) an entropy-based mosaic update method for coping with the variable visualization conditions during the procedure. We also implemented a blending method to generate a photo-realistic retina mosaic

**Results** Several experiments on retinal mosaicking in live on human (without laser at this moment) with a computer-assisted slit-lamp prototype will be presented

**Conclusion** Computer-assisted slit-lamp prototype could be usefull in a next future for retinal documentation and laser navigation

*Commercial interest*

• F031

**Evaluation of choroidal thickness depending on the stage of diabetic retinopathy by SD-OCT**

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**Purpose** To evaluate the choroidal thickness in diabetes and study its variation with different stages of diabetic retinopathy (DR).

**Methods** Retrospective study of 155 eyes of 83 diabetic patients treated between June 2011 and October 2012. All patients benefited from a complete ophthalmologic examination, laboratory tests conventional A-scan ultrasonography,choroidal and retinal tomography

**Results** The mean age patients was 57,54 with. There was not diabetic retinopathy DR(-) in 36,77% of cases. There was a minimal nonproliferative diabetic retinopathy NPDR in 17,41%, moderate NPDR in 15%, moderate preproliferative PPDR in 5,8%, severe PPDR in 11,6%, moderate PDR in 6,45%, severe PDR 4,51% and 2,58% in complicated PDR. The tomographic study objectified a thinner choroid in diabetic patients, with mean choroidal thickness (MCT=263+30 $\mu$ m) and subfoveal thickness (SFVCT=261,4+33,8 $\mu$ m). In the group with RD (+), choroid thickness (p=0,04), significantly decreased compared to DR (-) group, but not correlate with the severity of DR. There was, however, a statistically significant difference between the different stages of DR for the following variables: HBA1C, visual acuity and near VA (p<10-3). The SD-OCT has objectified a macular retinal thickening which increased significantly with the severity of DR (p<10-3). Moreover, the choroidal thickness varied little between the various stages of DR. We found a statistically significant correlation between SFVCT: duration of hypertension, the rate group. While the MCT was significantly correlated with ser

**Conclusion** SD-OCT now possible to explore the choroid, measure its thickness and assess its variation with the DR stages

• F030

**Phacoemulcification with intravitreal bevacizumab injection in patients with cataract and diabetic clinically significant macular edema**

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**Purpose** To evaluate efficacy of intravitreal injection of bevacizumab at the time of cataract surgery in patients with diabetic clinically significant macular edema.

**Methods** Eighteen diabetic patients, with clinically significant macular edema and cataract who have undergone phacoemulsification with intravitreal injection of 1.25 mg bevacizumab at the time surgery, were retrospectively evaluated. All patients were evaluated by spectral OCT before, 1 month, and 3 months after the surgery. The main parameters were the best-corrected visual acuity (BCVA) and central macular thickness (CMT). Paired samples t-test was used for statistical analysis.

**Results** The mean best-corrected visual acuity was  $0.16 \pm 0.07$  at baseline,  $0.63 \pm 0.44$  at 1 month and  $0.68 \pm 0.12$  at 3 months after the surgery. The BCVA levels recorded at 1 month and 3 months after the surgery were significantly higher than the initial BCVA ( $P = 0.001$ ). The mean CMT was  $384.4 \pm 102.4 \mu\text{m}$  at baseline,  $288 \pm 44.1 \mu\text{m}$  at 1 month and  $254.9 \pm 11.9 \mu\text{m}$  at 3 months after the surgery. The CMT values recorded at 1 month and 3 months after the surgery were significantly lower than the initial CMT ( $P < 0.001$ ,  $P < 0.001$ ).

**Conclusion** Phacoemulsification with intravitreal injection of bevacizumab at the time of cataract surgery provides a decrease in CMT with a gain in BCVA in diabetic patients with clinically significant macular edema and cataract.

• F032

**Association of OCT parameters with HbA1c level and diabetes duration in early stage of diabetic retinopathy**

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**Purpose** To investigate the association of the Fourier domain optical coherence tomography (FD-OCT, Optovue) parameters with the level of HbA1c and duration of the diabetes mellitus (DM) in patients at the early stage of diabetic retinopathy (DR).

**Methods** Thirty eyes of 30 patients with minimal DR (non-DR or mild NPDR) that were examined at the Chiba University Hospital from December 2011 to March 2012 were studied. The participants had no other ocular diseases and no history of surgeries. Three parameters embedded in the Optovue, the macular mapping 5 (MM5), RNFL, and ganglion cell complex (GCC), were performed, and the coefficients of correlation between these parameters and the HbA1c and the DM duration were analyzed with the Spearman rank correlation. A  $P < 0.05$  was considered significant.

**Results** All of the GCC parameters and RNFL parameters were not significantly correlated with both the HbA1c and the DM duration. The thickness of the outer-inferior area (mean= $275.9 \pm 18.1 \mu\text{m}$ ) was significantly correlated with the HbA1c ( $P=0.046$ ,  $R_s=0.371$ ). The outer-superior area (mean= $276.0 \pm 17.7 \mu\text{m}$ ) and the outer-temporal area (mean= $277.9 \pm 14.6 \mu\text{m}$ ) were significant correlation with the DM duration ( $P=0.015$ ,  $R_s=0.468$ ;  $P=0.022$ ,  $R_s=0.439$ , respectively).

**Conclusion** The significant positive-correlation between the retinal thickness of the outer area of the MM5 sector and HbA1c and DM duration may be due to a subclinical increase of vascular permeability. It might also be due to Müller cell swelling because Müller cell density is known to be higher in the outer area than the center area.

**Poster session 2 : Physiology-Biochemistry-Pharmacology / Retina-Vitreous / Neuro-ophthalmology-Strabismology-Paediatric Ophthalmology-History of Ophthalmology**

• F033

**Purtscher-like retinopathy associated with acute pancreatitis at Vilnius University Hospital Santariskiu Klinikos**

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**Purpose** To report two cases of Purtscher-like retinopathy associated with acute pancreatitis.

**Methods** Retrospective analysis of two cases of Purtscher-like retinopathy associated with acute pancreatitis. Fluorescein angiography (FAG), retinal photography, laboratory tests, computer tomography (CT) were analyzed.

**Results** 2 cases were identified in a period from 2007 to 2013. The first, 57 y.o patient with a history of recently treated acute pancreatitis, was referred to clinic 2 weeks after visual impairment. Visual acuity (VA) was 0.3 in the right eye and 0.25 in the left eye. FAG revealed prolonged arterio-venous passage time, enlarged foveal avascular zone (FAZ), fluorescein blockage temporal to optic nerve disc due to soft exudates. Patient was treated with systemic steroids intravenously. VA improved to 1.0 after 9 days of treatment. The second, 39 years old patient was referred to our clinic 3 days after visual impairment. At the time of hospitalization despite patients normal vital signs, laboratory tests and CT revealed acute pancreatitis and reactive hepatitis. VA was hand motion in the right eye and 0.01 in the left eye. FAG showed large FAZ and evidence of leakage from vessels in the late phase. After treatment with intravenous steroids, VA improved to counting fingers at 30 cm in the right eye and 0.03 in the left eye.

**Conclusion** Prognosis of Purtscher-like retinopathy may depend on systemic disease severity, VA in early stages and the presence of macular ischemia. Pancreatitis-associated retinopathy is rare and there are no evidence-based therapeutic recommendations.

• F034

**Branch retinal vein occlusion and vitreovascular traction: An SD-OCT case-control study**

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**Purpose** To determine the prevalence of vitreovascular traction at the obstruction site in patients diagnosed with branch retinal vein occlusion (BRVO).

**Methods** In this prospective observational case-control study thirty-two consecutive cases of BRVO were studied with spectral-domain optical coherence tomography (SD-OCT) to detect the presence of vitreovascular traction or vitreous adhesión at the occlusion site. The fellow eye was used as control.

**Results** SD-OCT directed to the occlusion site revealed a vitreovascular traction in eight eyes (25%). In fourteen eyes (43.75%) an adherent posterior hyaloid without signs of traction was found. Regarding to the same vessel segment of the fellow eye, none of the controls revealed vitreovascular traction, while vitreous adherence was present in 12 (37.5%). Vitreovascular traction at the occlusion site was significantly associated with BRVO ( $p=0.024$ , Chi-squared test).

**Conclusion** Vitreous traction at the occlusion site could play a role in the etiology of some cases of BRVO.

• F035

**Intravitreal ranibizumab following bevacizumab for macular edema due to retinal vein occlusion**

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**Purpose** Macular edema is the main cause of visual impairment secondary to retinal vein occlusions (RVO). Current treatment is based on intravitreal injections of corticosteroids or VEGF inhibitors. Bevacizumab was first used in the eye on an off-label basis, whereas ranibizumab was approved in this indication in January 2012 in France. Our work's objective was to analyse the becoming of patients who received bevacizumab then ranibizumab successively in management of RVO.

**Methods** This study includes the patients treated effectively with intravitreal bevacizumab for macular edema due to RVO and next treated with intravitreal ranibizumab. We included 9 patients presented with a central retinal vein occlusion (CRVO) and 6 patients with a branch retinal vein occlusion (BRVO). Ophthalmological examination performed after intravitreal injections consists in logMAR visual acuity and central macular thickness measurement by OCT.

**Results** Intravitreal injections of anti-VEGF allowed an improvement of the visual acuity and a reduction of the central macular thickness. Final visual acuity was better after treatment with ranibizumab than after bevacizumab in patients presenting with CRVO (the average gain of visual acuity was 0.30 logMAR). No infectious complication was noted.

**Conclusion** The therapeutic transition from bevacizumab to ranibizumab stabilizes both visual acuity improvement and decrease of macular thickness even in long time treated patients, despite vision impairment produced by old edema, worsening cataract, or apparition of retinal ischemia areas. In this short series of patients, replacement of bevacizumab by ranibizumab didn't have negative impact on visual results despite treatment was continued on a longer time course.

• F036

**Spectral-domain optical coherence tomography (SD-OCT) patterns in macular edema associated with retinal vein occlusion**

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Montfleury

**Purpose** To correlate spectral-domain optical coherence tomography (SD-OCT) characteristics of macular edema (ME) due to retinal vein occlusion (RVO) with mean best corrected visual acuity (BCVA).

**Methods** Fifty patients (50 eyes) having macular edema due to CRVO or BRVO, were retrospectively reviewed. Statistical analysis was used to evaluate the relative contribution of several variables, including SD-OCT characteristics such as baseline central retinal thickness (CRT), central intraretinal cyst size, serous retinal detachment (SRD) size, photoreceptor inner segment/outer segment (IS/OS) integrity and external limiting membrane (ELM) status with mean BCVA.

**Results** Thirty three patients had BRVO (66%), 14 patients (28%) had CRVO and 3 patients (6%) had HVO. Mean CRT was 574.52  $\mu$ m. There was a strong correlation between CRT and visual acuity ( $CCP=0.41$ ;  $P=0.003$ ). Cystoid macular edema was present in 46 eyes (92%) with central intraretinal cyst in 36 eyes (72%). Mean BCVA was statistically better in eyes without central intraretinal cyst ( $P=0.015$ ) but there was not a significant difference depending of the cyst size ( $CCP=0.28$ ;  $P=0.09$ ). SRD occurred in 22 eyes (44%) and mean thickness of SRD was 237  $\mu$ m. Mean BCVA was statistically better in eyes without SRD ( $P<0.001$ ) but there was not a significant difference depending of the cyst size ( $CCP=0.27$ ;  $P=0.21$ ). IS/OS line was disrupted in 17 eyes (34%) and absent in 21 eyes (42%). Eyes without IS/OS line showed worse BCVA ( $P<0.001$ ). Mean BCVA was better when IS/OS line was intact ( $P<0.001$ ). MLE was intact in 6 eyes (24%) over 25 patients. Mean BCVA was statistically better when MLE was not disrupted ( $P<0.001$ ).

**Conclusion** SD-OCT is an important tool in macular edema evaluation. Our results suggest that baseline SD-OCT characteristics, the status of photoreceptor IS/OS and ELM can be helpful in predicting the final visual outcome in patients with macular edema due to retinal vein occlusion.

**Poster session 2 : Physiology-Biochemistry-Pharmacology / Retina-Vitreous / Neuro-ophthalmology-Strabismology-Paediatric Ophthalmology-History of Ophthalmology**

• F037

**Hypertensive retinopathy complicated with bilateral retinal neovascularization: PRP versus intravitreal anti-VEGF treatment**

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**Purpose** To report a case of hypertensive retinopathy with bilateral retinal neovascularization treated with anti-VEGF intravitreal injection in one eye and panretinal photocoagulation (PRP) in the fellow eye.

**Methods** A 33-year-old male patient presented with gradual visual loss in both eyes for the last five months. From his medical history, idiopathic systematic hypertension was diagnosed five months ago. There was no other relevant medical or family history. Best corrected visual acuity (BCVA) was CF in the right eye and 8/10 in the left eye. Anterior segment examination was unremarkable on both eyes. Fundus examination showed abnormalities in both eyes compatible with hypertensive retinopathy and extensive areas of neovascularization. OCT examination showed no evidence of macular oedema. Fluorescein angiography (FFA) revealed macular ischaemia in the right eye and large areas of retinal ischemia and retinal neovascularization with vascular leakage, mainly across the temporal arcades, in both eyes. Indocyanine green angiography (ICG) showed no further pathology. Patient was treated with an anti-VEGF (ranibizumab) intravitreal injection in the right eye and PRP laser in the left eye.

**Results** Follow up examination one and two months post-operatively showed no change in BCVA in either eye. FFA documented regression of retinal neovascularization mainly in the right, while in the left eye there were still areas of vascular leakage across the temporal arcades.

**Conclusion** Hypertensive retinopathy can be rarely complicated with retinal neovascularization.

• F038 / 4714

**Intravitreal afibercept (IVT-AFL) for macular edema secondary to CRVO: Results of COPERNICUS and GALILEO studies**

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**Purpose** Evaluate efficacy and safety of IVT-AFL for treatment of macular edema due to CRVO.

**Methods** Patients randomized to IVT-AFL 2-mg (IVT-AFL 2q4) or sham monthly for 24 weeks. COPERNICUS: all patients received IVT-AFL PRN from week 24-100 (IVT-AFL 2q4 PRN and sham IVT-AFL PRN). GALILEO: IVT-AFL PRN was administered to IVT-AFL group from week 24-76 and sham group from week 52-76.

**Results** Proportion of IVT-AFL 2q4 PRN and sham IVT-AFL PRN patients gaining ≥15 letters was 56.1% vs 12.3% and 49.1% vs 23.3%, respectively, at week 24 and 100 (COPERNICUS) and 60.2% vs 22.1% and 57.3% vs 29.4%, respectively, at week 24 and 76 (GALILEO; all P<0.01). Mean change in BCVA was +17.3 vs -4.0 (P<0.0001) and +13.0 vs +1.5 ETDRS letters (P=0.007) at week 24 and 100 (COPERNICUS) and +18.0 vs +3.3 (P<0.001) and +13.7 vs +6.2 letters (P=0.007) at week 24 and 76 (GALILEO) in IVT-AFL 2q4 PRN and sham IVT-AFL PRN groups, respectively. Mean reduction from baseline CRT was 457.2 vs 144.8 μm (P<0.001) and 390.0 vs 343.3 μm at week 24 and 100 in COPERNICUS and 448.6 vs 169.3 μm (P<0.0001) and 389.4 vs 306.4 μm at week 24 and 76 in GALILEO in IVT-AFL 2q4 PRN and sham IVT-AFL PRN groups, respectively. Most frequent ocular SAEs in IVT-AFL 2q4 PRN group were cataract (COPERNICUS) and macular edema (GALILEO). APTC ATEs occurred only in COPERNICUS (2 (1.8%) IVT-AFL, 2 (2.7%) sham).

**Conclusion** IVT-AFL treatment resulted in early, sustained VA gains. Maintenance of VA gains with an extended treatment interval after initial monthly dosing depended on monthly monitoring. Long-term fixed dosing regimens may be a better treatment paradigm.

*Commercial interest*

• F039 / 4468

**Wide field imaging in Coat's disease**

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**Purpose** To report the usefulness of wide field imaging and angiography in patients with Coat's disease.

**Methods** In 5 consecutive patients, wide field imaging using scanning laser ophthalmoscopy and angiography (Optomap) was performed before treatment. The laser was performed on the basis of the wide field images. When the ophthalmologist considered that the treatment was complete, the imaging was repeated. After reviewing the images, the ophthalmologist decided whether further treatment was required.

**Results** Among all patients, wide field imaging enabled to monitor the localization of laser treatment with respect to the vascular abnormalities and non perfusion areas displayed on angiography. In all cases, additional laser treatment was performed on the basis of image lecture although the treatment had been considered to be complete by the ophthalmologist.

**Conclusion** Wide field imaging and wide field angiography enables to target laser treatment in Coat's disease.

• F040

**Optic disk changes in retinal vein occlusion and correlated factors**

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**Purpose** To evaluate optic disk changes in retinal vein occlusion (RVO) patients and to find risk factors related to increasing cup to disk ratio (C/D ratio)

**Methods** We used data collected in Korean RVO survey. It was retrospective, cross-sectional, nation wide and multicentral study. Data of RVO patients were collected from 41 clinical sites which joined in Korean RVO survey from April 2010 to December 2011. Patients' whole follow up data at base line, 3-month, 6-month, 9-month and 12-month were collected and analyzed. Among the data, we used fundus photographs (FDP), fluorescein angiogram (FA) and medical record at base line, 6-month and 12-month. C/D ratio was analyzed by three glaucoma specialists. FDP and FA findings including disk hemorrhage, disk edema, non-perfusion area, disk leak etc and medical records including intraocular pressure (IOP), intravitreal injection of bevacizumab (IVB), steroid therapy etc were analyzed for correlation to C/D ratio increasing.

**Results** Among enrolled 557 patients, 127 patients met the inclusion criteria. 57 patients were branch retinal vein occlusion (BRVO) and 73 patients were central retinal vein occlusion (CRVO). C/D ratio increased from 0.45 at base line to 0.52 at 12-month (p<0.001; paired T-test). Non-perfusion area, disk edema, disk hemorrhage and disk leak at base line and IOP at 12-month showed correlation to increase of C/D ratio (p=0.021, p=0.001, p<0.001, p=0.001, p=0.016 respectively). But IVB and steroid use were not related to increased C/D ratio. Detailed subgroup analysis (CRVO, BRVO) will be appeared on the presentation.

**Conclusion** Eyes with RVO showed increasing C/D ratio. Base line perfusion status and disk damage had correlations to increase of C/D ratio..

*Commercial interest*

**Poster session 2 : Physiology-Biochemistry-Pharmacology / Retina-Vitreous / Neuro-ophthalmology-Strabismology-Paediatric Ophthalmology-History of Ophthalmology**

• F041 / 3466

**Autofluorescence patterns and visual acuity in macular telangiectasia type 2**

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**Purpose** An analysis was performed with a view of determining potential associations between typical patterns seen on autofluorescence (AF) imaging in patients with Macular Telangiectasia (MacTel) type 2 and distance visual acuity at presentation as well as visual change over a 2-year follow-up period, thus ascribing predictive value to AF patterns in MacTel.

**Methods** A subgroup of 135 patients (229 eyes) enrolled in the MacTel study underwent AF imaging. Images were graded at the Moorfields Eye Hospital Reading Centre. Recorded AF patterns at baseline and at two years included the typical pattern of increased central AF due to loss of foveal masking, localized decreased AF at the end of a retinal vessel and a large area of decreased AF. Best-corrected visual acuity (BCVA) was measured by means of the Early Treatment for Diabetic Retinopathy Study charts at baseline and after two years. Statistical associations were derived by means of a generalized linear model.

**Results** Presence of increased macular AF ( $p=0.004$ ), a large area of decreased AF ( $p<0.001$ ) or decreased AF at the end of a retinal vessel ( $p<0.001$ ) at baseline were significantly associated with worse BCVA. Presence of increased macular AF ( $p<0.001$ ) or of localized decreased AF at the end of a retinal vessel ( $p<0.001$ ) and the absence of a large area of decreased AF ( $p<0.001$ ) were predictive of a subtle, but statistically significant drop in BCVA at 2 years.

**Conclusion** The typical pattern of increased central AF at baseline is encountered in patients with worse BCVA in MacTel type 2 and is also predictive of further subtle visual loss in a period of two years. Decreased AF is found in patients with worse baseline BCVA though no further visual loss at two years is observed.

• F042

**Response of retinal pigment epithelial detachments to intravitreal afibbercept in neovascular age-related macular degeneration refractory ranibizumab**

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**Purpose** Recent reports demonstrated that large retinal pigment epithelial detachments (PED) associated with neovascular age-related macular degeneration (AMD) was successfully treated with afibbercept. The aim of this study is to report unexpected short-term response of afibbercept in the treatment of AMD with associated large PED which had been failed to ranibizumab treatment.

**Methods** The method comprised a retrospective review of the medical records of patients with PEDs associated neovascular AMD and recently treated with afibbercept and previously treated with ranibizumab.

**Results** Two eyes of male Japanese patients of ages 79 and 80 years old with large serous PEDs and subretinal fluid (SRF) associated with occult choroidal neovascularization and neovascular AMD were treated with afibbercept after intravitreal ranibizumab had failed to resolve the lesions. All had complete resolution of SRF and complete or near-complete resolution of the serous PEDs after afibbercept injections over a 2-month period. However, after the second injection of afibbercept, all eyes displayed fibrovascular PED in the lesion where serous PED was observed. Moreover, one eye presented a sudden retinal hemorrhage and a recurrent PED just after the third injection of afibbercept, and the visual acuity decreased 10/20 to 2/20.

**Conclusion** Intravitreal afibbercept may be carefully applied for for serous PED in neovascular AMD patients after ranibizumab have previously failed. Larger studies with longer follow-up are required to determine the role of afibbercept in treatment of serous PED in neovascular AMD.

• F043

**AMD Drusenoid deposits characterization: Interest of OCT, OCT en FACE**

GONZALEZ C

FUTUROPHTA, Toulouse

**Purpose** To study AMD drusenoid deposits with OCT en Face and see all the input of this technique and software

**Methods** 204 eyes of 102 patients, 32 men, 70 women, with AMD drusenoid deposits (Cuticular drusen, Subretinal drusenoid deposits, soft Drusen, Drusenoid PED,Pseudodotilliform AMD). Deposits were evaluated by OCT, notably OCT en FACE (Spectralis HRA-OCT, spectral domainOCT). Autofluorescence, IR imaging, ETDRS visual acuity (VA), complete ophthalmic examination with Fundus exam were added. The size, characteristics, number, topography of the lesions, their growth way were evaluated, as well as their environment above and below (particularly IS-IS, plexiform layer, choriocapillaris structure and thickness). Each element was studied, compared cut to cut, layer to layer and time to time to itself and to each other data, every 4 months.

**Results** VA improved in 85% cases, stabilized in 15%. AF imaging and their region finder analyze were the main elements of the atrophic lesions study. The surface of the atrophic area grows by 17%, the edge changes in 20%. Speed growth was in average 1,15mm<sup>2</sup>/year. At OCT, thickness of photoreceptor, pigment epithelium layer diminished about 25% and 35% at the area's edge. Choriocapillary depth values, FA and ICG data were mainly significative in the large atrophic areas and less than AF indications. This protocol has a little impact on the evolution of atrophic areas, apparently less than monthly IVT, and the same as AMD by itself.

**Conclusion** The study of atrophic process and its progression is a main question in the AMD follow –up, the evaluation of the protocol treatments and their safety too.

• F044

**Neovascular AMD with high recurrences and atrophic areas - Potential relationship between neovascular complication and atrophy**

GONZALEZ C

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**Purpose** To evaluate the possible relationship between Neovascular complication and atrophy and each one characteristics input

**Methods** 144 eyes of 72 patients, 21 men, 51 women, 72 with retrofoveal neovascularisation complicating AMD, high recurrences, and atrophic areas,<sup>72</sup> contralateral eye with atrophic areas only. First and 2 months' interval follow-up exam included ETDRS visual acuity (VA), complete ophthalmic examination, fluorescein and infracyanine (ICG) angiography, optical coherence tomography (OCT), (HRA Spectralis-OCT), Autofluorescence imaging (FAF),( region finder software,to evaluate Atrophic areas). Protocol treatment: Series of 3 monthly Ranibizumab IVT, in loading dose and as Neovascular activity needed. Atrophic areas characteristics, their growth way were studied. The impacts of atrophy on high recurrence Neovascular AMD, and vice versa are evaluate, compared to own atrophy evolution and average Neovascular evolving way

**Results** VA improved in 34% cases, stabilized in 37%. Total Atrophic area surface was 0- 5mm<sup>2</sup> in 65%, (4, 898 mm<sup>2</sup>average),with moderate(1,817 mm<sup>2</sup>average)and slow (1,481 mm<sup>2</sup>/year average) growth.14% more in Neovascular eye. 24% more than low recurrences Neovascular AMD. Little correlation between Atrophy growth and number of Neovascular recurrences. Both are different process. Being together increase Neovascular risk and lower treatment response. This protocol had good functional results, with less IVT needed even if frequent recurrences, and a little impact on the evolution of atrophic areas

**Conclusion** Potential relationship between Neovascular and Atrophic process is a main question in the AMD follow –up, protocol treatments options,etiopathogenic knowledge and therapeutic prospects.

## • F045

**Clinical manifestation of retinal pigment epithelial tear after age-related macular degeneration treatment**

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**Purpose** To determine the risk factors for and responses of treatments after the development of retinal pigment epithelial (RPE) tears after treatment of exudative age-related macular degeneration (AMD)

**Methods** A retrospective, consecutive chart review was performed for all patients with exudative AMD treated with intravitreal anti-vascular endothelial growth factor (VEGF) antibody or photodynamic therapy (PDT) between March 2010 and January 2013. The main outcome measures were time from first injection until development of RPE tear, and pre-RPE and post-RPE tear visual acuity. The post-RPE tear visual acuity conservational interval was defined between the time RPE tear occurred and the time visual acuity was decreased 0.1(decimal) from pre-RPE tear visual acuity.

**Results** A total of 219 eyes were treated with intravitreal bevacizumab, ranibizumab or PDT. 10 eyes from 10 patients developed a RPE tear(4.6%). 3 of 10 eyes were polypoidal choroidal vasculopathy (PCV). The average RPE tear patients' age was 75.4 years which is statistically greater than the others(65.7 years)( $p=0.001$ ). 90%(9/10) of the RPE tears occurred within the first 12 weeks of treatment initiation. The number of patients conserving whose post-RPE tear visual acuity was 5(50%) and their visual acuity was conserved until about 4 months. However, all 10 patients had poor visual acuity within 1 year follow-up period.

**Conclusion** RPE tears can occur after treatments for exudative AMD in more elder patients. Visual acuity could be conserved at early period after RPE tear occurred. However, visual acuity finally decreased within 1 year in spite of continuous treatment.

## • F046

**Multispectral retinal image analysis (MRI) for the assessment of subretinal fibrosis in neovascular age-related macular degeneration (nAMD)**

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**Purpose** To investigate the use of MRI for quantitative characterisation of subretinal fibrosis secondary to nAMD.

**Methods** MRI images of the posterior pole were acquired over 4 months from 20 eyes including those with inactive subretinal fibrosis and those being treated with ranibizumab for nAMD. Changes in morphology of the macula affected by nAMD were modelled and reflectance spectra at the MRI acquisition wavelengths (507, 525, 552, 585, 596, 611 and 650nm) were computed using Monte Carlo simulation. Quantitative indicators of fibrosis were derived by matching image spectra to the model spectra of known morphological properties.

**Results** The model spectra were comparable to the image spectra, both normal and pathological. The key morphological changes that the model associated with nAMD were gliosis of the IS-OS junction, decrease in retinal blood and decrease in RPE melanin. However, these changes were not specific to fibrosis and none of the quantitative indicators showed a unique association with the degree of fibrosis. Moderate correlations were found with the clinical assessment, but not with the treatment program.

**Conclusion** MRI can distinguish subretinal fibrosis from healthy tissue. The methods used show high sensitivity but low specificity, being unable to distinguish scarring from other abnormalities like atrophy. Quantification of scarring was not achieved with the wavelengths used due to the complex structural changes to retinal tissues in the process of nAMD. Further studies, incorporating other wavelengths, will establish whether MRI has a role in the assessment of subretinal fibrosis in the context of retinal and choroidal pathology.

## • F047

**Choroidal thickness in dry age-related macular degeneration**ZABADANIK, ASCASO FJ, HERRERA L, PADGETTE, NUÑEZ E, CRISTOBAL JA  
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**Purpose** To compare the choroidal thickness (CT) in the macular area in eyes with nonexudative age-related macular degeneration (AMD) with that of unaffected fellow eyes and eyes of healthy controls.

**Methods** Enhanced depth imaging (EDI) was obtained by spectral-domain optical coherence tomography (SD-OCT) (Spectralis, Heidelberg-Engineering, Germany) in 47 eyes of 52 patients ( $78.7 \pm 7.1$  years) with AMD, 96 eyes of 96 healthy controls ( $74.0 \pm 8.5$  years) and in 5 unaffected contralateral eyes ( $68.6 \pm 14.4$  years). Eyes were divided into 3 groups: 47 eyes in group A (affected eyes with AMD), 5 eyes in group B (unaffected fellow eyes), and 96 eyes in group C (right eyes of age-matched controls). Choroidal thickness was measured from the posterior edge of the retinal pigment epithelium to the choroid-scleral junction in the subfoveal area (SCT) and 1 mm away from the fovea in the nasal (NCT) and temporal (TCT) regions. Statistical analysis was conducted to compare mean choroidal thicknesses.

**Results** In the AMD group the mean choroidal thicknesses were  $164.9 \pm 62.4$   $\mu\text{m}$  at the fovea (SCT),  $150.7 \pm 64.8$   $\mu\text{m}$  nasally (NCT), and  $171.1 \pm 63.3$   $\mu\text{m}$  temporally (TCT). In the unaffected fellow eyes these measurements were  $219.2 \pm 143$   $\mu\text{m}$ ,  $215.8 \pm 129.1$   $\mu\text{m}$ , and  $226.2 \pm 113$   $\mu\text{m}$ , respectively. Finally, the control group showed the following values:  $234.9 \pm 100.4$   $\mu\text{m}$ ,  $224.7 \pm 96.4$   $\mu\text{m}$ , and  $233.0 \pm 95.5$   $\mu\text{m}$ , respectively. Choroidal thickness did not show any statistically significant differences among the three groups of eyes when age was counted as a confounding variable ( $p>0.05$ , ANOVA test).

**Conclusion** There were no significant differences between choroidal thickness of nonexudative age-related macular degeneration with the contralateral unaffected eye or the control group.

## • F048

**Retinal tubulations in geographic atrophy associated with age-related macular degeneration**

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**Purpose** To describe the presence of retinal tubulations at the boundaries and within areas of retinal atrophy in patients with geographic atrophy (GA) associated with age-related macular degeneration (AMD) by spectral-domain optical coherence tomography (SD-OCT).

**Methods** 82 consecutive patients, 50 years or older with GA associated with AMD were examined between January 2012 and July 2012. Patients were evaluated by SD-OCT and fundus autofluorescence (FAF). Patients with single or multiple and well-defined areas of atrophy in whom retinal layers could be visualized were included. FAF was performed at the same time that OCT (Spectralis, Heidelberg Eng). Three acquisition protocols were performed: high-resolution horizontal B-scan for foveal study and two macular cubes to evaluate the whole macular surface (19 horizontal and 19 vertical B-scans centred on the fovea). Main Outcomes Measures: Presence of spherules/tubulations within the atrophic area in patients with GA associated with AMD.

**Results** 50/82 patients with GA presented with single or multiple areas of atrophy with identifiable retinal layers. Clearly visible and slightly hyper/hyporeflective round, rosette-like, spherule-shaped structures were localized in the outer nuclear layer (ONL). These structures occasionally contacted the external limiting membrane. They were adjacent to the margin between preserved retina and atrophic area and within the atrophic area. In these cases, uniformly hyporeflective, hyporeflective and isoreflective spherules surrounded by hyperreflective halo that might correspond with retinal tubulation were present in the same eye.

**Conclusion** Retinal tubulations can be observed in the ONL in patients with GA associated with AMD.

**Poster session 2 : Physiology-Biochemistry-Pharmacology / Retina-Vitreous / Neuro-ophthalmology-Strabismology-Paediatric Ophthalmology-History of Ophthalmology**

• F049

**Intercellular trafficking of Alu RNAs causes geographic atrophy expansion in AMD**

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**Purpose** Age-related macular degeneration (AMD) accounts for the bulk of blindness in the elderly in Western societies. The hallmark of advanced atrophic AMD, geographic atrophy (GA), is loss of retinal pigment epithelium (RPE) cells that expands centrifugally over time. We previously identified decreased abundance of the enzyme DICER1 and accumulation of its substrate Alu RNA in RPE cells as a cause of GA (Kaneko et al. Nature, 2011). We hypothesized that disease progression is due to transfer of cytotoxic Alu RNA from dying RPE to neighboring healthy cells by means of bleb/microvesicle RNA trafficking.

**Methods** Human RPE or 661W cells were transfected with DICER AS oligonucleotide. Blebs/exosomes were collected from conditioned media by differential centrifugation. RNA content of the isolated fractions was quantified by qPCR. For live cell imaging, FITC-Alu RNA transfected hRPE were stained with Fluorescent Cellvue®. Subretinally injected mouse eyes were stained (anti-ZO1), flatmounted and imaged with confocal microscopy.

**Results** Blebs and microvesicles isolated from DICER1-deficient RPE cells show increased abundance of Alu RNA. Additionally, exposing healthy RPE or photoreceptors to conditioned medium from Alu-enriched cells leads to cell death. Live cell imaging identified the presence of FITC-Alu RNA in Alu-naïve cells co-cultured with cells transfected with the fluorescently tagged RNA. Further, we demonstrate that subretinally injected vesicles induce RPE degeneration in mice.

**Conclusion** These data provide evidence for "neighborhood poisoning" as a mechanistic basis for progression of atrophic AMD. Targeting intercellular spread of Alu RNA holds therapeutic promise for inhibiting expansion of this devastating disease.

• F050

**New diagnostic and therapeutic strategies in acute and chronic central serous chorioretinopathy**

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**Purpose** Central serous chorioretinopathy (CSCR) is a disease in which a serous detachment of the neurosensory retina occurs over an area of leakage from the choriocapillaris through the retinal pigment epithelium (RPE). CSCR can be acute or chronic. Classic image studies for CSCR are Fluorescein Angiography (FA) and Optical Coherence Tomography (OCT). No medical therapy is currently indicated for CSCR despite a range of potential medical treatments evaluated in many case reports. Argon laser photoocoagulation can be considered only with a single leak located more than 300 µm from the center of the fovea. Autofluorescence (AF) with ultra-widefield scanning laser and Subthreshold Micropulse (SDM) photostimulation with true yellow 577nm diode laser are the newer diagnostic and therapeutic options.

**Methods** AF with ultra-widefield scanning laser (Daytona®, Optos plc UK) was performed in fifteen patients affected by acute and chronic CSCR. All the areas of serous retinal detachment visible at AF as hyperfluorescence were treated with SDM photostimulation (IQ 577™ true yellow laser, Iridex CA). Controls were made at 15 days, 1 and three months after the treatment.

**Results** Ultra-widefield AF allowed to identify several zones of serous detachment not detected with FA and out of range of OCT scans. In most of cases we obtained the complete resolution of serous detachment and visual acuity improvement. Gain in visual acuity was better in acute cases of CSCR.

**Conclusion** Ultra-widefield AF followed by SDM photostimulation can be an effective diagnostic and therapeutic option for patients with acute and chronic CSCR.

• F051

**Central serous chorioretinopathy in pregnancy: A report of two cases**

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**Purpose** Pregnancy has been implicated in the pathogenesis of central serous chorioretinopathy, along with stress, steroids, Cushing's syndrome, systemic hypertension and lupus erythematosus. We present two cases of central serous chorioretinopathy (CSC) during otherwise healthy pregnancies.

**Methods** Two patients that developed CSC during pregnancy are studied. Ophthalmological findings including visual acuity, slit-lamp biomicroscopy, fundus examination, fundus photography and optical coherence tomography are presented. Patients were re-evaluated six months after delivery.

**Results** Both patients had spontaneous resolution of CSC after delivery.

**Conclusion** CSC is a benign, self-limited disease that can present with or without white retinal exudates. Elevated levels of glucocorticoids, present during pregnancy, can contribute to the pathogenesis of this entity.

• F052

**Leopard-spot pattern of yellow-orange subretinal deposits in central serous chorioretinopathy**

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**Purpose** Central serous chorioretinopathy (CSC) is characterized by a serous detachment of the neurosensory retina caused by focal or diffuse dysfunction of the retinal pigment epithelium. A patient with a leopard-spot pattern of yellow-orange subretinal deposits as a rare dramatic form of CSC is described.

**Methods** A 65 year-old man with severe chronic obstructive pulmonary disease and congestive heart failure noticed a poor visual acuity after waking up from a coma elicited by an acute exacerbation of COPD and need for ventilation treatment. He underwent a full ophthalmic exam and extensive imaging including fluorescein angiography, OCT and the use of infrared and auto-fluorescent light.

**Results** In the ocular history there was prior cataract surgery because of severe subcapsular posterior cataract in both eyes. Visual acuity (VA) was reduced to counting fingers in the right eye and perception of light in the left eye. Fundoscopy showed a leopard-spot pattern of yellow-orange subretinal deposits in the posterior pole more pronounced in the right eye compared to the left. On FFA, the deposits were hypofluorescent with additional multiple pinpoint areas of hyperfluorescence spread over the posterior pole. Spectral OCT demonstrated a shallow detachment of the neurosensory retina with extensive intraretinal fluid and alternating atrophic areas and deposits at the level of the RPE which corresponded with hypo- and hyperfluorescent areas with autofluorescent light.

**Conclusion** Coarsely mottled or leopard-spot hyperpigmentation and secondary retinal detachment may develop as a rare dramatic complication in patients with CSC.

**• F053****Effect of intravitreal bevacizumab for chronic, recurrent, or atypical central serous chorioretinopathy**

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**Purpose** To evaluate the efficacy of intravitreal bevacizumab injection (IVB) for the treatment of chronic, recurrent, or atypical central serous chorioretinopathy (CSC).

**Methods** Forty-nine eyes of 49 patients who were diagnosed with chronic, recurrent, or atypical CSC and treated with intravitreal injection of 1.25 mg/0.05 ml bevacizumab, were included for this retrospective study. Best-corrected visual acuity (BCVA) and central macular thickness (CMT) were analyzed at baseline, 3, 6, 9, and 12 months after initial IVB.

**Results** Patients received the mean of  $2.16 \pm 1.34$  IVB during the mean follow-up period of  $11.8 \pm 7.4$  months, and 20.4% of patients had recurrent episode after initial resolution of subretinal fluid in this period. The mean baseline BCVA was  $0.27 \pm 0.26$  logarithm of minimum angle of resolution, and the mean baseline CMT was  $353 \pm 129$  um. BCVA was significantly improved to  $0.16 \pm 0.18$  ( $P = 0.005$ ) and  $0.14 \pm 0.12$  ( $P = 0.000$ ) at 3 and 6 months, respectively. However, the improvement of BCVA was not significant at 9 months and 12 months. CMT was significantly reduced throughout the follow-up period and  $216 \pm 101$  um ( $P = 0.008$ ) at 12 months.

**Conclusion** Intravitreal bevacizumab injection may be an effective treatment option for patients with chronic, recurrent, or atypical CSC. Further prospective, long-term studies are warranted.

**• F054****Choroidal thickness in eyes with idiopathic epiretinal membrane**

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**Purpose** To compare the choroidal thickness in the macular area in eyes with an idiopathic epiretinal membrane (ERM) with that of unaffected fellow eyes and eyes of healthy controls.

**Methods** Enhanced depth imaging (EDI) was obtained by spectral-domain optical coherence tomography (SD-OCT) (Spectralis, Heidelberg-Engineering,Germany) in 66 eyes of 50 patients ( $73.91 \pm 11.1$  years) with ERM and 96 eyes of 96 healthy controls ( $74.04 \pm 8.5$  years). Eyes were divided into 3 groups: 66 eyes in group A (affected eyes with ERM), 36 eyes in group B (unaffected fellow eyes), and 96 eyes in group C (right eyes of age-matched controls). Choroidal thickness was measured from the posterior edge of the retinal pigment epithelium to the choroid-scleral junction in the subfoveal area (SCT) and 1 mm away from the fovea in the nasal (NCT) and temporal (TCT) regions. Statistical analysis was conducted to compare mean choroidal thicknesses.

**Results** In the ERM group the mean choroidal thicknesses were  $241.1 \pm 98.1$   $\mu\text{m}$  at the fovea (SCT),  $225.7 \pm 88.7$   $\mu\text{m}$  nasally (NCT), and  $232.6 \pm 85.6$   $\mu\text{m}$  temporally (TCT). In the unaffected fellow eyes these measurements were  $216.4 \pm 72$   $\mu\text{m}$ ,  $213.3 \pm 73.7$   $\mu\text{m}$ , and  $214.8 \pm 71$   $\mu\text{m}$ , respectively. Finally, the control group showed the following values:  $234.9 \pm 100.4$   $\mu\text{m}$ ,  $224.7 \pm 96.4$   $\mu\text{m}$ , and  $233.0 \pm 95.5$   $\mu\text{m}$ , respectively. Choroidal thickness did not show any statistically significant differences among the three groups of eyes when age was counted as a confounding variable ( $p > 0.05$ , ANOVA test).

**Conclusion** No relationship between the presence of an ERM and changes in choroidal thickness was found. Epiretinal membrane is a disease caused by changes in the vitreous humor. Choroid does not seem to participate in the pathogenesis of this disorder.

**• F055****Topographic distribution of vascular and neurodegenerative signs in type 2 idiopathic macular telangiectasia**

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**Purpose** Idiopathic macular telangiectasia type 2 (MacTel) is associated with characteristic vascular signs including leakage/staining on fluorescein angiography (FA) as well as neurodegenerative signs including a progressive loss of luteal pigment. The distribution of luteal pigment can be assessed by dual-wavelength autofluorescence (DWAF) imaging. Our aim was to investigate the relationship of vascular and neurodegenerative signs of type 2 MacTel in FA and DWAF images.

**Methods** FA and DWAF images were acquired using a Heidelberg Spectralis SLO and a HRA Classic SLO device respectively. Thirty eyes of 30 MacTel patients were examined. Images were aligned to attain exact correspondence. Staging of luteal pigment loss in DWAF images was performed according to the system developed by Zeimer et al. The extent of abnormalities in the two imaging modalities was compared.

**Results** According to severity of luteal pigment loss, 6 eyes were at stage 1, 14 at stage 2 and 10 at stage 3. At stages 2 and 3, the area with early FA changes was always smaller and contained within the area of luteal pigment loss. This also held true for stage 1 except in very early cases with minimal pigment loss. In these, FA changes, especially late staining appeared more extensive. Dense pigment plaques appeared as masking dark areas in both FA and DWAF images with hyperfluorescent spots in DWAF. Subretinal neovascularization broke up the pattern in 3 eyes.

**Conclusion** Our results indicate that in non-neovascular type 2 MacTel, luteal pigment loss appears more extensive than vascular change. The relative time sequence of changes may provide clues to the primary underlying cause. Confirmation on a larger sample with follow-up is indicated.

**• F056****Choroidal thickness changes determined by SS OCT following intravitreal bevacizumab**

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**Purpose** To report choroidal thickness (CT) changes using a SS OCT prototype following intravitreal bevacizumab injections.

**Methods** Seventeen patients were treated by one single intravitreal 1.25 mg injection of bevacizumab (IVB). The macular area was examined using an SS-OCT prototype system based on swept-source OCT and was used to image the full-thickness choroid and sclera. A CT profile of the macula was created by manually measuring CT (from the posterior edge of retinal pigment epithelium to the choroid/sclera junction) subfoveally, and three further determinations were performed every 750  $\mu\text{m}$  temporal and nasal to the fovea. CT was determined immediately before and 24 hours after performing 1.25 mg IVB.

**Results** Seventeen eyes from 17 patients (11 eyes with exudative age related macular degeneration, 4 eyes with retinal vein occlusion, 1 eye with myopic choroidal neovascularization, and 1 eye with diabetic macular edema) were examined. Nine patients were male and 8 were female. Mean age was  $73 \pm 11$  years range 53 to 88. Pearson correlation coefficient among observers averaged 0.90 (range 0.54 to 0.99). Changes in choroidal thickness were not significant for any of the determinations (Student t test for paired data). Changes in central foveal thickness were almost statistically significant (Student t test for paired data).

**Conclusion** Our findings suggest that one single IVB does not seem to induce acute choroidal thinning.

**Poster session 2 : Physiology-Biochemistry-Pharmacology / Retina-Vitreous / Neuro-ophthalmology-Strabismology-Paediatric Ophthalmology-History of Ophthalmology**

• F057 / 3467

**Evaluation of ultra-widefield fundusautofluorescence in non-infectious posterior uveitis**

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**Purpose** Posterior uveitis comprises a heterogenous group of diseases with inflammatory alterations of the posterior fundus and is a common cause of visual impairment and blindness. The goal of this study is to evaluate the diagnostic value of wide-field-fundusautofluorescence (FAF) of patients with non-infectious posterior uveitis and chorioretinal alterations.

**Methods** 73 eyes from 51 patients were included. Best-corrected visual acuity, wide-field color and FAF images achieved by a wide-field SLO (Optomap P200Tx, Optos PLC, Dunfermline, Fife, Scotland, UK) and a full ophthalmological examination were obtained from each patient. A systematic analysis of chorioretinal alterations detected with FAF and color images was conducted followed by the evaluation of the diagnostic information of wide-field FAF compared to the clinical finding and wide-field color images respectively.

**Results** 52 of 73 included cases showed peripheral alterations. In 32 cases, wide-field FAF images revealed a greater number and more extended chorioretinal alterations than corresponding wide-field color images of the posterior fundus.

**Conclusion** In this study, wide-field FAF images showed more chorioretinal alterations than seen in funduscopic or in color SLO images. Therefore, wide-field FAF images offer important additional information in detection and documentation of peripheral and central chorioretinal alterations.

• F058 / 4467

**Swept source optical coherence tomographic findings in a case of bilateral macular coloboma**

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**Purpose** To describe the findings of macular coloboma using swept-source optical coherence tomography (SS-OCT)

**Methods** Case report

**Results** A 35-year-old man was found to have bilateral deep excavation of the maculae and was diagnosed clinically with bilateral macular coloboma. SS-OCT showed deep excavation of the sclera corresponding to the area of coloboma. There is loss of overlying retinal pigment epithelium (RPE), neurosensory retina and choroid within the area of excavation. A thin membranous structure is seen overlying the area bared sclera. At both edges of the coloboma, intraretinal cystic changes can be seen.

**Conclusion** The SS-OCT is able to image the posterior border of the deep coloboma in this case. In addition, the detailed structure of the retinal layers can be studied. This membranous structure likely represents the remnants of degenerative neurosensory retina and internal limiting membrane. In addition, we also demonstrated intraretinal cysts, not previously reported, at the edges of the coloboma. The use of SS-OCT technology has enabled the capturing of the retina, RPE and choroid at a much better resolution and this has served to provide greater understanding of the anatomical changes seen in macular coloboma.

• F059

**Retinal arteriolar diameter response to flicker light provocation - A useful marker for risk stratification in cardiovascular disease?**

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**Purpose** To evaluate the benefit of bilinear and linear fitting to characterize the retinal vessel dilation to flicker light stimulation for the purpose of risk stratification in cardiovascular disease.

**Methods** Methods Forty-five patients (15 with coronary artery disease (CAD), 15 with Diabetes Mellitus (DM) and 15 with CAD and DM) all underwent contact tonometry, digital blood pressure measurement, fundus photography, retinal vessel oximetry, static retinal vessel analysis and continuous retinal diameter assessment using the retinal vessel analyser (and flicker light provocation). In addition we measured blood glucose (HbA1c) and keratinin levels in DM patients.

**Results** Results With increased severity of cardiovascular disease a more linear reaction profile of retinal arteriolar diameter to flicker light provocation can be observed.

**Conclusion** Absolute values of vessel dilation provide only limited information on the state of retinal arteriolar dilatory response to flicker light. The approach of bilinear fitting takes into account the immediate response to flicker light provocation as well as the maintained dilatory capacity during prolonged stimulation. Individuals with cardiovascular disease however show a largely linear reaction profile indicating an impairment of the initial rapid dilatory response as usually observed in healthy individuals.

• F060

**Variation of online Amsler grid from mobile apps, YouTube and Google**

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**Purpose** To analyse and evaluate the dimensions and instructions of Amsler grid from three sources: Mobile Phone Apps, YouTube and Google.

**Methods** Searches were performed for Amsler grid in the three areas. Mobile Apps were viewed on a smartphone with 8.9 cm screen. Grids were measured on a 33.7cm computer screen with 1368 x 768 resolution. Grids were assessed for number of squares, size, viewing distance and instructions on use. Calculation of subtended visual angle was made where possible.

**Results** 10 apps and 7 videos were identified. Over 2 million Amsler grids were found from Google and the first 100 grids were chosen. Black grid on white background design was used in 72%, 11% with white-on-black and other formats in 17%. Instructions about monocular testing, distortion and scotoma detection were described in 96%, 97% and 89% respectively. Frequency of testing was only stated in 24%, of which most advised between daily to weekly (75%) and up to monthly (1%). 66% of the grids were presented as 20x20, range from 8x8 to 30x30. Viewing distance was specified in 51%, range 30–40cm. The average calculated retina projection was 12.3° (range 6.8° to 21.4°). Only two grids would project to 20° of the macula. Google and YouTube are freely available and Mobile Apps can cost up to £28.99. Most of the sources are produced by ophthalmologist practices (39%) followed by Optometrist related groups (16%).

**Conclusion** Video-sharing media and Internet may be an effective way to disseminate the test for patients. Our study shows there is wide variation in the charts that are available which may affect the result of self monitoring tool.

**Poster session 2 : Physiology-Biochemistry-Pharmacology / Retina-Vitreous / Neuro-ophthalmology-Strabismology-Paediatric Ophthalmology-History of Ophthalmology**

• F061

**HMGB1 is a potent inducer of choroidal angiogenesis through a MyD88-dependent manner**

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High-mobility group box 1 (HMGB1) is an endogenous alarm sensor against extracellular damage signals and initiates TLR (toll-like receptor) responses closely linked to angiogenesis through MyD88, its specific adaptor protein. Interest in regulation of HMGB1-TLR as a potential therapeutic approach is increasing but this regulatory effect in pathologic angiogenesis still remains unclear. Through a mouse model of laser coagulation-induced choroidal neovascularization (CNV) we confirmed that disturbing HMGB1 by its specific antibody and recombinant HMGB1 protein significantly reduces and induces CNV, respectively. To examine whether HMGB1 influences CNV through TLR signal, we then used MyD88 KO mice and the effects of HMGB1 antibody and recombinant protein on CNV completely blocked. Taken together, these data suggest that HMGB1 is a potential endogenous or exogenous regulator of pathophysiological angiogenesis and MyD88 is an essential mediator for HMGB1-induced angiogenesis.

• F062 / 3655

**Refractive error after intravitreal bevacizumab for threshold disease in retinopathy of prematurity: Two-years follow-up**

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**Purpose** To examine the association between myopic refractive error at two years of life in infants who postnatally received an intravitreal bevacizumab injection or standard laser therapy for threshold retinopathy of prematurity (ROP) in fundus zone I or zone II.

**Methods** In the retrospective non-randomized interventional comparative study, infants who consecutively received a single intravitreal bevacizumab (0.375 mg) injection (study group) were compared with infants who had previously undergone standardized retinal argon laser therapy (control group). The follow-up examination included cycloplegic refraction.

**Results** The study group consisted of 7 children (13 eyes) and the control group included 14 children (27 eyes). Both groups did not differ significantly in gestational age and follow-up. At the end of follow-up of 24 months, refractive error (median:+1.00 diopters (D) (+4.0D to -10.00D) versus median:-7.50D (+2.50D to -15.0D); $P=0.001$ ), astigmatism (median:0.25D (0D to 2D) versus median:1.25 D (0D to 5.75D); $P=0.001$ ) and prevalence of high myopia ( $\leq-6D$ ) (15±10% versus 52±10%; $P=0.04$ ) were significantly less in the study group than control group. After adjusting for gestational age, follow-up time, gender and birth weight, refractive error ( $P=0.007$ ), astigmatism ( $P=0.01$ ) and prevalence of high myopia ( $P=0.03$ ; Odds Ratio:0.13) were significantly associated only with laser therapy versus bevacizumab therapy.

**Conclusion** A single intravitreal bevacizumab injection as compared to conventional retinal laser coagulation was associated with a lower degree of myopization and less astigmatism at two year follow-up.

• F063 / 3656

**Wide field imaging in patients treated with vigabatrin**

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**Purpose** Vigabatrin is an active treatment for epilepsy in children particularly for West's syndrome. In children when visual field testing cannot be performed, regular ERG recording is mandatory to screen for retinal toxicity. Some peripheral retinal changes have been described with ophthalmoscopy. The purpose of this study was to evaluate the occurrence of retinal changes wide field imaging and to compare the results with ERG recordings.

**Methods** Five consecutive patients treated with vigabatrin were followed up with Flicker ERG and wide field imaging using scanning laser ophthalmoscopy (Optomap, Optos). Flicker ERG amplitude was considered as abnormal below 50µV.

**Results** Two patients had abnormal ERG recordings and presented with peripheral pigmentary changes. One patient presented with abnormal flicker ERG and normal retinal imaging. Two patients had normal ERG recordings with no peripheral changes on wide field imaging.

**Conclusion** The follow-up with ERG in children treated with vigabatrin is complicated because the recordings are regularly repeated. A follow-up protocol combining electrophysiological recordings and wide field imaging could simplify screening for retinal toxicity but the remains to be demonstrated with a prospective study.

• F064 / 3657

**Utility of systematic ophthalmological screening in congenital toxoplasmosis: Epidemiological study of a French cohort between 1990 and 2011**

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**Purpose** Ophthalmologic complications of congenital toxoplasmosis, such as retinochoroiditis, are particularly feared. Any child with confirmed congenital toxoplasmosis is treated and regularly followed with many fundus examinations. The aim of our study is to describe the management and monitoring of a cohort of patients with congenital toxoplasmosis in Alsace, and the impact of this disease in terms of parental anxiety using a standardized questionnaire.

**Methods** Our study recorded 35 children with congenital toxoplasmosis, born between 1990 and 2011 in Alsace. All patients were followed by an ophthalmologist. A standardized questionnaire concerning the experience of pregnancy and post-natal follow up were submitted to parents.

**Results** At birth, a retinochoroiditis was detected in 2 children, and only one child developed the disease during routine monitoring. Brain abnormalities were noted in 3 children at birth, none of them presented with retinochoroiditis to this day. An average score of 15 out of 23 was found by our standardized questionnaire, reflecting significant anxiety due to congenital toxoplasmosis.

**Conclusion** Parental anxiety due to congenital toxoplasmosis is obvious. A directed follow-up by a complete pediatric examination at birth, including eye fundus, and good information on functional signs of ocular toxoplasmosis may improve the screening without impact on visual function.

**Poster session 2 : Physiology-Biochemistry-Pharmacology / Retina-Vitreous / Neuro-ophthalmology-Strabismology-Paediatric Ophthalmology-History of Ophthalmology**

• F065 / 3658

**Quality of life among children with severe and chronic ophthalmic conditions**

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**Purpose** To evaluate the impact on the quality of life of children from 8 to 18 years-old of a rare and serious ophthalmological condition (cataract and congenital glaucoma).

**Methods** Analysis of the answers obtained between May and November, 2012 by autoquestionnaire (KIDSCREEN-10) among children from 8 to 18 years-old and from their parents and approaching their physical, social, moral and educational wellness. The children are patients of a department of pediatric ophthalmology of the South of France and followed-up on the long term for chronic and severe ophthalmological conditions.

**Results** 50 children (mean age 12 years) and 45 parents participated in this study. We register a lack of energy and an impression to be decreased to the everyday life to 32% of the children according to their parents (n: 14) and 27% (n: 13) according to the children. A feeling of sadness is present according to the parents for 43% of the staff (n: 19) and to 36% of the children according to their statements (n: 18). The solitude is felt at 64% (n: 28) of children according to their parents against 30% (n: 15) according to the children. The school life is affected according to the parents for 82% of the children (n: 37) and 70% when we question the children (n: 35). The global appreciation of the child's health is degraded at 30% of the children (n: 13) according to their parents against 22% according to the children (n: 11).

**Conclusion** Chronic ophthalmological conditions have certain noxious impact on the quality of life of the children and particularly in their social relationships and their school life. This study shows a good correlation between the felt of the parents on the real-life experience of their children and the appreciation which make the children themselves.

• F066

**Ocular morpho-functional analysis: Comparison between prematurely-born and born at term children**

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**Purpose** To evaluate the differences in ocular morpho-functional parameters between preterm and born at term children

**Methods** 50 eyes of 25 prematurely-born children (gestational age ≤34 weeks and birth weight ≤ 1495g) were compared to 40 eyes of 20 children born at term. The age at examination was 6-8 years old. In preterm group 18 eyes had retinopathy of prematurity (ROP): 8 were affected by mild ROP (stage 1-2) and 10 by severe ROP (stage 2-3), treated with laser photocoagulation. The children were submitted to a complete ophthalmic evaluation including ETDRS visual acuity (VA), contrast sensitivity (CS), pattern visual evoked potential (pVEP), retinal nerve fiber layer thickness (RNFL, disc area) and central macular thickness (CMT) measured by spectral domain optical coherence tomography (SD-OCT)

**Results** No statistically significant differences were found in functional or structural data between prematurely-born and born at term children except higher values for disc area in preterm. Conversely many statistically significant differences (VA, CS, P100 amplitude, disc area and CMT) emerged between control and preterm group affected by ROP, especially in severe ROP

**Conclusion** Many differences in ocular morpho-functional parameters are pointed out between full term and preterm children affected by ROP. The most important dissimilarities are in stage 3. Maybe it can be correlated with treatment's damage or due to incomplete development of optic pathways. Because the principal differences are in functional parameters (VA, CS, VEP), without significant morphological changes, we hypothesize that impairment of vision in severe ROP is due not only to retinopathy of prematurity but also to brain damage.

• F067

**Choroidal thickness measurement in children using optical coherence tomography**

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**Purpose** To measure choroidal thickness in children of various ages by using spectral optical coherence Tomography with enhanced depth imaging (EDI). The primary outcome was to measure choroidal thickness in children. The secondary outcomes were to investigate the association between subfoveal choroidal thickness and ocular axial length, age, gender, weight, and height in children.

**Methods** Hospital-based cross-sectional study. Healthy children visiting at the University Hospital of Besançon were prospectively included between May and August 2012. Optical coherence tomography with the EDI system (Spectralis®, Heidelberg, Germany) was used for choroidal imaging at nine defined points of the macula of both eyes. Axial length was measured by using IOL Master® (Carl Zeiss. Meditec. USA). Height, weight and refraction were recorded.

**Results** Three hundred forty height eyes from 174 children were imaged. The mean age of the children studied was  $8.70 \pm 2.89$  years (mean  $\pm$  SD); range 3.5-14.9 years. The mean subfoveal choroidal thickness in right eyes was  $341.96 \pm 74.7$   $\mu$ m. Mean axial length was  $22.30 \pm 1.05$  mm. Choroidal thickness increased with age ( $r=0.24$ ,  $p=0.017$ ), height and weight but not with sex ( $p>0.05$ ). It was also inversely correlated to axial length ( $r=0.24$ ,  $p=0.001$ ). There was a moderate correlation between the two eyes in terms of choroidal thickness ( $r=0.6$ ). The nasal choroid appeared thinner than in the temporal area (ANOVA,  $p<0.0001$ ).

**Conclusion** In children, choroidal thickness increases with age and is inversely correlated to the axial length. There is a significant variation of the choroidal thickness between children of the same age.

• F068

**Definition of a normative basis for peripapillary nerve fibers thickness in childhood with sd-oct**

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**Purpose** Since its inception, optical coherence tomography (OCT) has radically changed our way to practice ophthalmology. Yet, the analysis of databases of various machines has no value for patients under 18 years old. In this work we define a normative basis of the thickness of peripapillary nerve fibers in children with SD-OCT.

**Methods** We randomly selected 99 children (192 eyes) aged from 2 to 17 years. They were found to be healthy subjects with no history of eye disease and normal visual acuity. A study of the optic nerve head was conducted on Cirrus HD OCT. Two scans were performed in both eye to judge the good reproducibility of the measurement. We divided the population into three age groups (Group 1: 3-7 years / Group 2: 7-12 / Group 3: 12-18 years) before setting the average thickness of each quadrant.

**Results** Group 1 included 10 patients (19 eyes). The results reflected an overall thickness of  $100 \mu\text{m} \pm 8$  to the following distribution: temp:  $77 \mu\text{m} \pm 8$  / Upper:  $130 \mu\text{m} \pm 20$  / nasal:  $66 \mu\text{m} \pm 10$  / lower:  $131 \mu\text{m} \pm 12$ . Group 2 included 39 patients (74 eyes). The results reflected an overall thickness of  $99 \mu\text{m} \pm 8$  to the following distribution: temp:  $72 \mu\text{m} \pm 9$  / Upper:  $126 \mu\text{m} \pm 16$  / nasal:  $70 \mu\text{m} \pm 13$  / lower:  $128 \mu\text{m} \pm 16$ . Group 3 included 50 patients (99 eyes). The results reflected an overall thickness of  $97 \mu\text{m} \pm 11$  for the following distribution: temp:  $63 \mu\text{m} \pm 14$  / Upper:  $125 \mu\text{m} \pm 16$  / nasal:  $72 \mu\text{m} \pm 15$  / lower:  $128 \pm 19$ .

**Conclusion** The definition of a normative database in children is essential on OCT because it's integrated into the daily practice of the ophthalmologist. We define RNFL in children under 18 years old and find a very slight change over time, mainly in the temporal quadrant.

**• F069****Functional vision of young adults with type i diabetes since childhood**

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**Purpose** To evaluate vision of young adults with type 1 diabetes (T1D) since childhood.

**Methods** During 1989-90 the prevalence of diabetic retinopathy (DR) was evaluated in a population-based cohort of 5-16-year-old children with T1D living in the catch-area of the Northern Ostrobothnia Hospital District, Finland. These 216 individuals were contacted 18 years later and evaluated for best corrected visual acuity (BCVA) LogMar, contrast sensitivity (CS) and stage of retinopathy. The CS test was performed using Vistech Chart at five spatial frequencies, 1.5, 3.0, 6.0, 12.0 and 18.0 cycles per degree (cpd). Retinopathy was diagnosed from fundus photographs, and data on past laser treatment and vitreoretinal surgery were also collected.

**Results** 106 patients took part in the study. There were 65 males (61%) and 41 females (39%) aged 30±3 years. The duration of diabetes was 23±4 years. Two of the subjects were blind in the right eye. The CS was evaluated of 210 eyes of 106 patients. It was normal in all cpds studied in 93 of 210 eyes, or 39 of 106 patients, and abnormal in one or several cpds in 117 eyes. Abnormality was most likely to be found at cpd 6.0; this was the case in 37% of the eyes. BCVA was 0.1 (LogMar) or better in 189/210 eyes. 34 patients had proliferative DR (PDR). CS was impaired in 30/34 (88%) of patients with PDR and 37/72 (51%) without PDR ( $p<0.001$ ).

**Conclusion** The majority of young adults with T1D had good BCVA. However, CS was outside normal range in more than one half, and particularly in patients with PDR.

**• F070****The socio-economic status of young adults with type 1 diabetes since childhood**

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**Purpose** The aim of the study was to evaluate the socio-economic status of young adults with type 1 diabetes (T1D) since childhood with particular interest in its relation to the severity of diabetic retinopathy (DR).

**Methods** During 1989-90 the prevalence of DR was evaluated in a population-based cohort of 5-16-year-old children with T1D living in the catch-area of the Northern Ostrobothnia Hospital District, Finland. These 216 individuals were contacted 18 years later and evaluated for retinopathy as well as asked to fill out a socio-economic status questionnaire.

**Results** 136 subjects (78 men) aged 30±3 years with median T1D duration of 23 years took part in the study. Proliferative DR (PDR) was diagnosed in 42 (31%) their mean age being 31±3 years. 97 (71%) were married, 31 (23%) single and 8 (6%) divorced. 61 subjects (45%) had a total of 123 children. A university degree was held by 12 (9%), that from a university of applied sciences by 45 (33%), 61 (45%) had finished vocational school, 10 (7%) were full-time students while 6 (4%) had received no education after comprehensive school. A full-time job was held by 66 subjects (72%) with no or non-PDR and 24 (57%) with PDR; 4 (4%) vs. 6 (14%) subjects were unemployed, respectively ( $p=0.071$ ), and none vs. 5 (12%) were on pension, respectively. The patients with PDR were more likely to be outside working life ( $p<0.001$ ), but equally likely to have a spouse ( $p=0.683$ ) and children ( $p=0.457$ ).

**Conclusion** The majority of young adults with T1D have an active role in the society with work or study, spouse, and raising children. A third of the patients had PDR, and although they had similar educational level and families, they were more likely not to be taking part in working life than those with less severe DR.

**• F071****Differential diagnostic of eye morphometric parameters in patients with refractive and axial myopia**

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**Purpose** Many authors have shown that myopia is associated with changes in the anatomical parameters of the eye. The aim: To study morphometric parameters in patients with refractive and axial myopia

**Methods** The observation involved 264 patients (502 eyes) with varying degrees of myopia (myopic refraction of -2.75 diopters), age - 18.41 year. Patients were divided by type of myopia: refractive (RM) - 133 eyes, axial - 217 eyes, mixed (SM) - 91 eyes, and combined - 61 eyes. Methods: visometry for distance, keratometry and refractometry after cycloplegia with cyclomed 1% with Accuref-K 9001, Shin Nippon, the definition of the horizontal pupil diameter by keratotopograf PCT-110, Optopol and horizontal corneal diameter by KR-8900 Topcon and HRK-7000 Huvitz.

**Results** Results Analysis of the data showed that the axial myopia has significantly larger average diameter , the radius and refraction of cornea, pupil diameter , length of the vitreous humor, axis length of the eye and the degree of myopia, compared with refractive myopia. Significantly higher mean values of intraocular pressure and the width of the anterior chamber angle were established in refractive myopia in comparison with axial myopia ( $p < 0.01$ ). Values of anterior chamber depth and lens thickness between patients with axial and refractive myopia showed no significant differences ( $p > 0.1$ ).

**Conclusion** Obtained results showed that morphometric parameters of the eye, intraocular pressure in patients with axial and refractive myopia have practical value for early detection and prediction of the development and progression of myopia in patients with myopia

**• F072****Changes in protein composition of tear fluid in schoolchildren with high progressing myopia**

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**Purpose** Defining the protein composition of tear fluid (TF) of schoolchildren with high progressive myopia

**Methods** We tested TF samples of 35 patients aged 10-17 (ave. 13.1±2.2 yrs) with myopia from 5.0 to 20.5D (ave. 9.5±3.7D) and year progression rate 0.98±0.8D. 19 had various forms of peripheral vitreochoreoretinal dystrophies. No patients used contact lenses to correct myopia. The control group contained TF samples of 9 children aged 10-13 (ave. 12.4±0.5 yrs) with normal eye fundus. Total protein content (TPC) was determined according to M.Bradford. Qualitative determination of protein composition and content in TF was performed by SDS polyacrylamide gel electrophoresis. The proteins were separated by unidirectional gel electrophoresis and stained with coomassie solution. Qualitative determination of major proteins – lactoferrin (Ltf) and lysozyme (Lys) – was conducted using two different primary antibodies (sandwich ELISA). Variation significance of qualitative parameters was assessed by nonparametric methods using Mann-Whitney U-test.

**Results** Characteristic distinctions were found for TF of highly myopic children: 1) a significant reduction of TPC against the norm: 5.65 [4.85; 6.33] mg/ml and 7.66 [6.83; 8.16] mg/ml respectively ( $p=0.000408$ ); 2) a significant increase of Ltf/TPC ratio against the norm: 0.23 [0.2; 0.29] and 0.37 [0.33; 0.43] resp.,  $p=0.000185$ . The lysozyme share in the total protein content (Lys/TPC) was invariable.

**Conclusion** The data confirm the pathogenetic role of the damaged antioxidant protective system of eye media and tissues in the development of myopia. Reduced TPC and increased share of Ltf in TF may be considered as possible diagnostic signs of the progressing character of myopia.

**Poster session 2 : Physiology-Biochemistry-Pharmacology / Retina-Vitreous / Neuro-ophthalmology-Strabismology-Paediatric Ophthalmology-History of Ophthalmology**

• F073

**Epidemiology of patients undergoing strabismus surgery at adult age: Retrospective study of 221 patients**

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**Purpose** The strabismus in adult patients could be either the persistence of misalignment since the infancy before the age of visual maturation, or as a consequence of neurological, traumatic or orbital pathology. The aim of this study is to characterize the patients population undergoing strabismus surgery at adult age.

**Methods** In this retrospective and monocentric study were reviewed 221 patients aged of 18 years and above; the time frame was 3 years. For each patient we analyzed different parameters : age, sex, the treated deviation, the performed surgery, the etiology of the deviation and the presence of diplopia or amblyopia.

**Results** Our patients, aged of 18 to 82 years, reported an onset of strabismus during childhood in 62% (50% with no previous surgical treatment, 20% with a recurrence of already treated strabismus and 30% with a consecutive deviation); 75% of them suffered of amblyopia. The cause of strabismus in 32% of our patients was a secondary deviation due to cranial nerve palsy (55%), traumatism (30%) or orbital pathology (20%). Exotropia was more frequent in our population than esotropia (40% versus 30%). Diplopia was reported in 12% of the patients. The surgery was performed on one eye in 90% and the most frequent operation was on horizontal rectus muscles (64%).

**Conclusion** Adult patients are most frequently suffering of infancy strabismus. When the strabismus onsets at adult age, the most common causes were paralytic strabismus followed by traumatic strabismus. Exotropia was the most prevalent deviation. Surgery could be a simple monocular intervention on rectus muscles or more complex in some cases. The adult patients undergoing strabismus surgery represent a heterogeneous population.

• F074

**Screening of visual disorders among high school students without expressed complaint**

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**Purpose** Many students underestimate their visual discomfort, although it may have an educational impact. We studied the prevalence of visual disorders among students without self-reported complaints.

**Methods** Four hundred students between 15 and 22 years of age responded to a questionnaire followed by a visual screening (refraction and binocular vision) in order to detect any visual discomfort that they might be unaware of. When visual problems were detected, the participants were suggested to have an ophthalmology and orthoptic assessment.

**Results** Visual disorders were found in 346 students. Thirty-two percent of them agreed to have an ophthalmology and orthoptic assessment. Best-corrected visual acuity was 20/20 in more than 95% of them. Nearly 60% were hyperopes, from which more than two thirds required an optical correction. Over or under-corrected myopia and uncorrected astigmatism were uncommon. Convergence insufficiency was found in 80%, and only a few ophthalmologic pathologies were diagnosed.

**Conclusion** Many students have vision problems to which they are accustomed. It was rare to find underlying ophthalmologic pathologies (strabismus, nystagmus, ...). In most cases, these functional symptoms are due to under-corrected hyperopia, possibly associated with convergence insufficiency. As visual demand is lower during primary education, some visual discomfort may only become symptomatic during the following years. However, the nature of these disorders suggests that they may interfere with education. We are now investigating the prevalence of learning difficulties among these participants, and whether there is an improvement after intervention.

• F075

**Prevalence of binocular anomalies among preschool children in Mashhad, Iran**

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**Purpose** To determine the prevalence of binocular anomalies among preschool children in Mashhad, Iran

**Methods** In a cross-sectional study with random cluster sampling, children aged 4 to 6 years old from kindergartens of Mashhad in Iran were selected. Examinations included: visual acuity, objective and subjective refractions, cover test, near point of convergence and stereopsis. Best corrected visual acuity worse than 8/10 or more than two Snellen lines difference between the eyes was defined as amblyopia. Anisometropia was defined as spherical equivalent refraction difference of 1.0 diopter and more between two eyes.

**Results** Of the 3765 selected children, 98.3% of them participated in the study. The mean age of the subjects was 5.09 (range: 4-6) years and 51.3% of them were boys. Strabismus was found in 1.2 % of the children and intermittent exotropia had the highest prevalence. Prevalence of amblyopia and anisometropia were 0.5% and 6% respectively. Heterophoria was found in 62.7% and shift was toward esophoria. The mean near point of convergence was  $5.09 \pm 1$  cm. Stereopsis of 100 sec/arc and better was found in 94.6% of the subjects.

**Conclusion** The results of this study indicated that the prevalence of strabismus was similar to other studies in Iran while there was a smaller prevalence of amblyopia. For preventing the incidence of binocular anomalies in children, a careful planning of the basic information is required to check the status of binocular vision.

• F076

**Change in the antioxidative capacity of extraocular muscles in patients with exotropia**

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**Purpose** We compared the oxidative stress and antioxidant capacities of the medial rectus muscles (MRMs) of control subjects and those with exotropia.

**Methods** A total of 40 MRMs from patients with exotropia and 40 MRMs from normal donor eyes (controls) were assessed. The mRNA levels of neuronal nitric oxide synthase (nNOS) and superoxide dismutase (SOD) were compared between the two groups using semi-quantitative reverse transcription-polymerase chain reaction. In addition, SOD1, SOD2, and lipofuscin levels were compared between the exotropic and control groups.

**Results** nNOS mRNA level was significantly higher in the exotropic group than in the control group ( $p < 0.001$ ). Western blot analysis revealed that SOD1 and SOD2 expression levels were higher in the control group than in the exotropic group ( $p < 0.05$ ). The relative lipofuscin fluorescence intensity was higher in the exotropic group than in the control group ( $p < 0.001$ ).

**Conclusion** The MRMs of patients with exotropia had a redox imbalance status. Further study is needed to investigate whether this imbalance in antioxidant capacity is present in the extraocular muscles of other patients with strabismus.

**Poster session 2 : Physiology-Biochemistry-Pharmacology / Retina-Vitreous / Neuro-ophthalmology-Strabismology-Paediatric Ophthalmology-History of Ophthalmology**

• F077

**Incidence of associated other oculomotor disturbances with internuclear ophthalmoplegia**

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**Purpose** Internuclear ophthalmoplegia (ION) is a central oculomotility disorder resulting from dysfunction of the medial longitudinal fasciculus. It is frequently associated with skew deviation and vertical nystagmus. We aimed to determine the incidence of oculomotility disorders associated with ION.

**Methods** Retrospective chart review of patients diagnosed with ION. Patients were retrieved from the Neuro-Ophthalmology Database of the Hôpital Ophtalmique Jules-Gonin. Patients' demographics, etiologies, uni-bilaterality, association with other oculomotility disorders were studied.

**Results** Our study comprised of 69 patients, 38F/31M, aged 16 to 77 years (mean 47 years). Etiologies included multiple sclerosis (MS, 39/69), stroke (20/69), tumor (5/69), and inflammatory/idiopathic (5/69). ION was unilateral in 39 cases and bilateral in 30 cases. Oculomotility disorders other than ION were found in 56 patients : upbeat nystagmus (31/56), downbeat nystagmus (29/56), oculo-cerebellar syndrome (25/56), skew deviation (18/59), VI palsy (7/56), III palsy (6/56), dorsal midbrain syndrome (4/56), and one-and-a-half syndrome (3/56). Stroke was responsible for the majority of skew deviation cases, whereas MS was the predominant cause of all the other oculomotility disturbances.

**Conclusion** More than 80% of patients with ION exhibited also other central oculomotility disturbances. Vertical nystagmus and oculomotor cerebellar syndrome outnumbered skew deviation. Other disturbances included VI nerve palsy, III nerve palsy, Parinaud's syndrome and Fisher's one-and-a-half syndrome. ION is rarely isolated and the spectrum of other oculomotor abnormalities is wide.

• F078

**Optical coherence tomography as a progression marker in mild cognitive impairment and Alzheimer's disease**

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**Purpose** To study the role of optical coherence tomography (OCT) as a progression marker in patients with mild cognitive impairment (MCI) and Alzheimer's disease (AD).

**Methods** We studied 36 eyes of 18 patients meeting criteria for AD (6 patients with mild AD, 7 patients with moderate AD and 5 patients with severe AD), and 21 eyes of 42 patients meeting criteria for MCI. In these two groups, we evaluated cognitive impairment through the Mini-Mental State Examination (MMSE), the verbal fluency test (VFT) and the clock test (CT) and we measured peripapillary retinal nerve fiber layer (RNFL) thickness, macular volume and thickness using OCT.

**Results** There is a significant correlation between the decrease in peripapillary RNFL thickness, macular volume and macular thickness and the severity of the cognitive impairment measured by the three neuro-psychological tests ( $p=0.00$ ).

**Conclusion** The reduction in peripapillary and macular thickness and macular volume is related to the severity of cognitive impairment. Thus, OCT can be used for the diagnosis of AD and MCI (especially in the diagnosis of early-stage AD), as well as to evaluate cognitive impairment progression.

• F079

**Evaluation of optical features of the macula in multiple sclerosis**

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**Purpose** Differences in optical and irregularity measures of abnormal retinal tissue may provide additional information of impairment of the retinal nerve fiber layer (RNFL) and ganglion cell/inner plexiform layer complex (GCL+IPL) in multiple sclerosis (MS). The purpose of our study was to estimate these retinal changes in MS.

**Methods** Twenty-seven patients with MS were examined using Stratus OCT. The raw macular OCT data was exported and processed using OCTRIMA software and the fractal dimension (FD) and layer index (LI) values of seven intraretinal layers were obtained. The enrolled eyes were divided into two groups, based on ON in the history (ON+ group, n=13 and ON- group, n=14). Data of 73 healthy subjects (N) were used as controls. ANOVA with Newman-Keuls post-hoc analysis was used for the comparison of FD and LI values. The level of significance was set at  $p<0.001$ .

**Results** A significant decrease was observed in LI in the entire macula and the perifoveal region in RNFL ( $12.4\pm1.4$ ,  $10.8\pm0.8$ ,  $9.3\pm1.4$  and  $14.0\pm1.7$ ,  $12.0\pm0.9$ ,  $10.0\pm1.7$  for the N, ON- and ON+ groups, respectively). The RNFL in the ON- group was significantly different from both the N and ON+ groups ( $p<0.001$  for all comparisons). No significant changes were found in the other layers. A significant increase was observed between the ON- and ON+ groups in FD in the entire macula and the perifoveal region in the RNFL layer ( $1.8\pm0.04$ ,  $1.8\pm0.07$   $p<0.001$  and  $1.6\pm0.06$ ,  $1.6\pm0.1$   $p<0.001$ , respectively) but no significant changes were found in the other layers.

**Conclusion** In MS, the optical features of the ganglion cells and the RNFL also change besides the pathological remodeling of macular tissue. This result may help to improve the diagnostic efficacy of OCT in MS.

**Commercial interest**

• F080

**Visual evoked potentials, short wave-length automated perimetry, standard automated perimetry, contrast sensitivity and stereoacuity testing in visually asymptomatic eyes of patients with multiple sclerosis**

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**Purpose** To evaluate the efficacy of PVEP, standard automated perimetry, short-wavelength automated perimetry, CS, stereoaucuity in detecting subclinical visual impairment in patients with multiple sclerosis (MS) and no history of optic neuritis.

**Methods** Twenty-seven MS patients (average age  $33.81\pm9.33$  years) underwent PVEP with 60 and 15-minute arc check sizes, SAP (Humphrey 750-II, SITA standard strategy), SWAP (program central 30-2), CS, Stereoacuity by RSA test. They were age and sex matched with twenty seven normal subjects.

**Results** Stereoacuity was significantly reduced in MS group compared with controls. ( $p=0.007$ ). P100 latency time for both check sizes was significantly prolonged in MS group compared with controls ( $p=0.005$ ). P100 amplitude significantly reduced in MS group compared with controls ( $p<0.001$ ). When comparing MS patients with the control group significant differences were found for SAP MD, SWAP MD, SAP PSD, SWAP PSD ( $p<0.001$ ). By considering predefined criteria, asymptomatic MS patients have shown abnormal stereoacuity in 22.2%, abnormal VEP in 40.7%, abnormal CS in 37%, abnormal SAP in 44% and abnormal SWAP in 37% of patients.

**Conclusion** Our results concluded the probable presence of subclinical visual impairment among MS patients without optic neuritis history and no visual symptoms. The Pattern VEP and perimetry test together are useful choices in the regular assessment of these patients.

**Poster session 2 : Physiology-Biochemistry-Pharmacology / Retina-Vitreous / Neuro-ophthalmology-Strabismology-Paediatric Ophthalmology-History of Ophthalmology**

• F081

**Neuromyelitis optica (devic's disease): The use of the spectral domain optical coherence tomography (OCT) in the follow up of the disease**

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**Purpose** To report a case of neuromyelitis optica (Devic's disease) which followed up with the use of optical coherence tomography (spectral domain OCT)

**Methods** A 24 year old female patient presented with vision deterioration due to acute onset of unilateral optic neuritis in her left eye (OS). She was referred to the neurologic department of our hospital for further assessment and the diagnosis of Devic's disease was set by the presence of anti - NMO - IgG antibodies. Patient's follow - up included visual acuity measurements, visual fields testing and electrophysiological evaluation with pattern visual evoked potentials (PVEP) according to ISCEV standards. Peripapillary RNFL thickness was assessed by the spectral domain OCT.

**Results** Visual acuity was measured at 1.0 at her right eye and 0.4 at her left eye. Visual field of her left eye showed a characteristic scotoma due to optic neuritis. The latency of the P100 was delayed demonstrating dysfunction of the optic nerve pathway. Thinning of the RNFL layer was documented in her left eye at OCT imaging. During follow up of the patient there was no further deterioration of the RNFL thickness despite recurrence of the optic neuritis three times.

**Conclusion** OCT is a valuable and sensitive tool in evaluating peripapillary RNFL in patients with neuromyelitis optica (vóοοc Devic).

• F082

**Retinal nerve fibre layer thickness as a neurological dysfunction marker in fibromyalgia patients**

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**Purpose** To assess retinal nerve fiber layer (RNFL) thickness in patients affected by fibromyalgia.

**Methods** 39 patients diagnosed with fibromyalgia and 39 age-matched healthy controls underwent a complete neuropsychiatric and ophthalmologic examination including best-corrected visual acuity, contrast sensitivity test, and the new Nsite Axonal Analytics application of the spectral domain Optical Coherence Tomography (SD-OCT). Statistical analysis was conducted to compare RNFL thicknesses between patients and controls.

**Results** RNFL thickness was significantly reduced in fibromyalgia patients ( $94.2 \pm 16.8$ ) compared with healthy controls ( $102.9 \pm 10.2$ ) in the mean values ( $p=0.007$ , Student t test), the ratio (cases- $0.9 \pm 0.3$ , controls- $1.1 \pm 0.3$ ,  $p=0.022$ ), the superonasal (cases- $99.3 \pm 20.3$ , controls- $116.4 \pm 16.7$ ,  $p<0.001$ ), inferonasal (cases- $96.7 \pm 28.5$ , controls- $126.2 \pm 25.9$ ,  $p<0.001$ ) and inferotemporal quadrant (cases- $134 \pm 26.9$ , controls- $144.7 \pm 19$ ,  $p=0.048$ ).

**Conclusion** Fibromyalgia patients showed a reduction of peripapillary RNFL thickness evaluated by SD-OCT. These findings, which have never been document in patients with fibromyalgia, suggest that neuronal degeneration could be present in the retina of fibromyalgia patients as previously observed in other neurodegenerative disorders.

• F083

**Optical coherence tomography and electrophysiology to evaluate Parkinson disease severity**

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**Purpose** To evaluate correlations between visual evoked potentials (VEP), pattern electroretinogram (PERG), and macular and retinal nerve fiber layer (RNFL) thickness measured by optical coherence tomography (OCT) and the severity of Parkinson disease (PD).

**Methods** Forty-six patients diagnosed with PD were enrolled in this study and underwent VEP, PERG, and Cirrus and Spectralis OCT measurements of macular and RNFL thicknesses, and evaluation of PD severity using the Hoehn & Yahr scale to measure PD symptom progression, the Schwab and England Activities of Daily Living Scale (SE-ADL) to evaluate patient quality of life (QOL), and disease duration. Logistic regression was performed to analyze which measures, if any, could predict PD symptom progression or effect on QOL.

**Results** Visual functional parameters (best corrected visual acuity, mean deviation of visual field, PERG P50 and N95 component amplitude, and PERG P50 component latency) and structural parameters (Cirrus and Spectralis OCT measurements of RNFL and retinal thickness) were decreased in PD patients compared with healthy controls. OCT measurements were significantly negatively correlated with the Hoehn & Yahr scale, and significantly positively correlated with the SE-ADL scale. Based on logistical regression analysis, foveal thickness provided by Cirrus OCT predicted PD severity, and QOL and amplitude of the PERG N95 component predicted a lower SE-ADL score.

**Conclusion** Patients with greater damage in the RPE or in the ganglion cells tend to have a lower QOL and more severe PD symptoms. Foveal thicknesses and the PERG N95 component provide good biomarkers for predicting QOL and disease severity.

• F084

**Relevance of contrast sensitivity for the diagnosis and monitoring of early stages of Alzheimer's disease**

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**Purpose** Several visual tests can be used to determine the behavior of neurons, including the contrast-sensitivity test (CST), which analyzes the actions of retinal ganglion cells and their cortical processes. Brain atrophy, one of the characteristic signs of Alzheimer's Disease (AD), is caused by severe loss of neurons in the brain, including several areas known to be involved in visual processing. Our aim was to analyze CST changes in early AD in order to ascertain whether or not CST could be used for AD diagnosis and progression control of the disease.

**Methods** Twenty GDS 4 AD patients, and 25 controls were examined at the Hospital Clínico San Carlos in Madrid (Spain) and underwent a complete ophthalmologic exam (visual acuity, refraction, CST, color test, biomicroscopy, IOP, and fundoscopy). The CST used was CSV-1000 E chart test, providing for four rows of sine-wave gratings, which test the spatial frequencies of 3, 6, 12, and 18 cycles/degree. The chart was presented at 2.5 m.

**Results** In AD patients, the CST proved lower in all spatial frequencies, the greatest decrease affecting the highest frequency.

**Conclusion** In early AD, the CST registered a decline in the function of both magnocellular and parvocellular retinal ganglion cells. The CST could be a useful tool for early diagnosis and follow-up of AD patients.

• F085

**Retinal layer segmentation: Non-invasive technique demonstrating hyperplasia of nerve fibers and ganglion cells in the Ataxia of Charlevoix-Saguenay**

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**Purpose** To present a new retinal layer segmentation technique, in order to evaluate the existence of nerve fiber hyperplasia in patients with the autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS).

**Methods** This observational cross-sectional study included five patients with a molecular diagnosis of ARSACS and 5 sex and age-matched healthy controls underwent a full ophthalmologic examination, which included a new technique to segment the retinal layers using Optical Coherence Tomography. Images and data were correlated with diffusion tensor colour encoded MRI maps, diffusion tensor tractographies, and retinal anatomopathological analysis.

**Results** Every patient showed increased thickness in the internal layers of the retina (inner limiting membrane, nerve fiber layer and ganglion cell layer) in OCT evaluation. These findings suggest the presence of neurofilamentous hyperplasia in the retina of patients with ARSACS and correlated with anatomopathological findings.

**Conclusion** We found evidence of ganglion cell and nerve fiber hyperplasia in the retina of ARSACS patients. Thus, a revision of the aetiopathogenic mechanisms is necessary in this disease.

• F086

**Optic disc drusen and peripapillary subretinal neovascular membranes in children**

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**Purpose** Choroidal neovascular membranes are a rare cause of decreased vision in children with optic disc drusen. It can cause central vision loss by subfoveal progression of the choroidal neovascularization, serous macular detachment, or submacular hemorrhage.

**Methods** We report two cases of optic nerve head drusen in childhood. A 6 year-old girl and a 12 year-old boy were both referred to our hospital for optic disc edema with unilateral visual loss.

**Results** Fundus examination showed pseudopapillary edema in the two cases. A pediatric check-up with neurologic and cardiovascular exams and CT scan were performed. B-Scan ultrasonography showed the presence of the optic nerve head drusen in both eyes but wasn't identified on CT scan in the two cases. In our cases, optic disc drusen causes peripapillary haemorrhages proceeding from the same mechanism with a juxtapapillary choroidal neovascularization. Because of the location of the hemorrhage only monitoring was recommended. Partial spontaneous visual acuity improvement in 6 months was observed with regression of haemorrhage.

**Conclusion** Optic nerve head drusen are calcified deposits arising from the prelaminar portion of the optic nerve, they have a reported prevalence of 0.4% in children. Although usually benign, their presence can sometimes be associated with serious ocular conditions and cause amblyopia. They have been reported to cause myriad local vascular complications, including central retinal artery and vein occlusion and peripapillary choroidal neovascularization.

• F087

**Accuracy of fluorescein angiography (FA) based diagnosis for retinopathy of prematurity: Expert versus non expert graders evaluation**

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**Purpose** To evaluate accuracy and inter-rater reliability of RetCam fundus images and digital camera fluorescein angiography (FA) images in acute retinopathy of prematurity (ROP) by comparing diagnoses given by trainee ophthalmologists with those provided by expert ophthalmologists.

**Methods** We conducted a multicentre retrospective observational study of diagnostic data from 60 eyes of 30 premature infants (average gestational age was 27 weeks, average birth weight 803 g) with classical ROP, stage II evaluated by RetCam 3 (Clarity, Pleasanton, CA) and fluorescein angiography. A staging grid with ocular fundus divided into 3 concentric zones and into 24 of 15° sectors centred around optic papilla was superimposed on 360° retina photomontages (Photoshop) acquired from RetCam and FA images. Non-expert agreement with expert diagnoses was measured for each sector by means of Cohen's kappa (Fleiss, 1981).

**Results** A high degree of concordance was found. Inter-rater agreement expert versus non-expert interpretations was really greater for fluorangiographic images than for RetCam ones with  $0.61 < k < 1$  for 58.5% of RetCam examined sectors and for 89.9% of FA examined sectors.

**Conclusion** FA images appear to be really clearer to interpret than RetCam images also by non-expert ophthalmologists. Our result confirms that FA is very good examination technique with a high degree of reliability, even where trainee practitioners are involved. ROP management can be improved by entrusting diagnostic responsibilities to trainee ophthalmologists, in order to extend access to correct diagnoses, recognition of threshold lesions and prompt treatment.

• F088

**A case of cortical blindness due to cerebral isquemia during OPCAB surgery and later recovery**

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**Purpose** To describe a case of cortical blindness caused by hypoxia during a bilateral lung transplant in a 19-year-old patient affected with cystic fibrosis and later recovery.

**Methods** 19-year old male diagnosed with cystic fibrosis is set to be operated of a bilateral lung transplant by OPCAB surgery. After the procedure the patient referred marked visual loss (hand movement). Slit lamp exploration was normal as well as fundoscopy, OCT, IOP and pupillary reflexes, finding severe alterations in the visual fields performed.

**Results** The patient was diagnosed with bilateral occipital ischemia confirmed by MRI and caused by cerebral hypoxia during surgery. During the last 4 weeks visual acuity has improved to 0.2 (OD) and 0.4 (OS). Visual field results have also improved. As of today the patient evolution is still being followed.

**Conclusion** The critical importance of reducing as much as possible the time of surgery when OPCAB techniques are performed and the possibility of clinical improvement patients may show after cortical isquemia.

**Poster session 2 : Physiology-Biochemistry-Pharmacology / Retina-Vitreous / Neuro-ophthalmology-Strabismology-Paediatric Ophthalmology-History of Ophthalmology**

• F089

**Adult-onset aqueductal stenosis: A case report**

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**Purpose** The authors describe a case of an adult-onset aqueduct stenosis.

**Methods** A 37-year-old man presented with a progressive blurring of vision in his left eye for a few days. He reported a fever episode two weeks before. Visual acuity was 10/10 P2 OD and 7/10 P2 OS. Pupils were equal without afferent defect. Neurologic examination was normal. Fundoscopic examination revealed a left optic disc oedema and a mild swelling of the right disc margins. Visual field examination demonstrated a bilateral enlargement of the blind spot associated with an infero-nasal defect on the left eye. Computed tomography showed enlargement of the third and lateral ventricles suggestive of aqueduct stenosis, which was confirmed by MRI. Ultimately we learned that the patient's mother presented an aqueduct stenosis too. Otherwise the febrile episode was linked to infectious mononucleosis attested by serology. Following endoscopic third ventriculostomy, there was an visual acuity improvement to 10/10 P2 OD and 9/10 P2 OS. Visual field was normal on the right eye, but infero-nasal defect remained on the left eye.

**Results** Hydrocephalus secondary to aqueduct stenosis may present at any age, however it is more frequent in childhood. Stenosis can be attributed to different causes. In three quarters of cases, etiology remains unknown. Clinical course may vary according to the age of the patient. In our case age of onset is atypical, as symptomatology, consisting of isolated visual disturbances. The unilateral infero-nasal visual field defect turns out to be also uncommon.

**Conclusion** In view of bilateral optic disc oedema, clinicians have to hint at the possibility of hydrocephalus secondary to aqueduct stenosis, even if clinical manifestations are not much suggestive.

• F090

**Presumed eosinophilic granuloma of the orbital roof in an adult man**

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**Purpose** To report on an unusual case of presumed adult-onset eosinophilic granuloma of the orbital roof, which showed spontaneous resolution.

**Methods** Descriptive review of the clinical records of one patient.

**Results** A 42-year-old man presented with a 3-month history of relapsing edema in his right upper eyelid. Visual acuity was 20/20 bilaterally. Apart from the detection of a group of dilated vessels in the right lower bulbar conjunctiva, both eyes were unremarkable. There was no proptosis and ocular motility was normal. CT and gadolinium-enhanced MRI head scans disclosed a 15 mm solid mass in the right anterior cranial fossa, which had eroded the orbital roof, penetrated into the orbit, and caused mild displacement of the levator palpebrae and superior rectus muscles. An initial diagnosis of intradiploic meningioma of the orbital roof was made. Total resection of the lesion was planned and the patient was put in the waiting list for neurosurgery. One year later, preoperative MRI head scans disclosed a significant regression of the lesion; consequently, neurosurgery was postponed. Six months later, MRI scans showed complete disappearance of the solid mass and full reossification of the orbital roof. A presumptive, final diagnosis of solitary eosinophilic granuloma of the orbital roof was made. He was cancelled from the waiting list for neurosurgery and MRI at 6-month intervals has been scheduled.

**Conclusion** Persistent upper eyelid edema may be the presenting sign of an osteolytic intracranial mass eroding the orbital roof and extending into the orbit. Radiological differential diagnosis may be difficult; in this report, the detection of an osteolytic lesion and its subsequent spontaneous resolution are suggestive of an adult-onset eosinophilic granuloma.

• F091

**Eye and optic tract function in patients with cavernous sinus meningioma undergoing CyberKnife robotic radiosurgery**

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**Purpose** To assess 6-month radiation-related ocular side effects in patients undergoing CyberKnife Robotic Radiosurgery which enables delivery of beams of high dose radiation with extreme accuracy. Ocular side effects after radiation therapy have been reported and include: dry eye syndrome, eyelash loss, cataract formation, retinopathy, corneal changes, neovascular glaucoma, optic neuropathy.

**Methods** We examined 9 patients undergoing CyberKnife Robotic Surgery for meningiomas located close to optic chiasm in order to assess effects of radiation on optic tract function. Patients received 18 Gy in 3 fractions. Doses delivered to optic tract were on average 15 Gy in 3 fractions. Ocular structures involve extremely radiosensitive tissues such as natural lens as well as relatively resistant tissues: cornea, sclera and optic nerve. Slit lamp biomicroscopy with dilated fundus exam, Best Corrected Visual Acuity (BCVA), Intraocular Pressure (IOP), Endothelial Cell Count (ECD), cataract formation, Retinal Nerve Fiber Layer (RNFL) and Central Macular Thickness were obtained at a baseline and six months after radiotherapy.

**Results** No pathological changes in slit lamp examination with dilated fundus exam were revealed. No significant cataract formation or progression was observed. No statistically significant changes in measured parameters regarding eye, optic nerve and optical tract were observed ( $p>0.05$  Wilcoxon paired test).

**Conclusion** Our study did not show any significant influence of radiation to eye and optic tract in patients treated with CyberKnife Robotic Radiosurgery for brain lesions in six month follow up period. Further observation of potential radiation-related ocular complications is required.

• F092

**Glaucomatous optic neuropathy in congenital glaucoma**

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**Purpose** Morphometric analysis of posterior pole structures of the eye in children with congenital glaucoma (CG)

**Methods** 34 children aged 3 months-11 yrs with various forms and stages of CG were examined with HRT-3 and Spectralis HRA+OCT.

**Results** We established the early appearance of deep and bulk excavation and reduction of neuroretinal rim area and volume at the beginning of glaucoma manifestation, mean total RNFL thickness reducing, reflecting the degree of the optic nerve structures atrophy. Correlations between changes of the optic disc parameters, glaucoma stages and the eye size were revealed. In all glaucoma eyes we noted thinning of peripapillary RNFL. 89% of children showed a decrease of arteries caliber (8-25% of normal) and veins (23-40% of normal). In 80% of the eyes there was an increase in AVI (ave 0.81). The choroidal topography study showed an increase of the subfoveolar thickness (305-957  $\mu\text{m}$ ) in 80% of children. Examination of the macular area revealed OD-OS asymmetry of total macular volume in glaucoma eyes (healthy eyes -  $8.50 \pm 0.36 \text{mm}^3$ ; glaucoma eyes -  $7.51 \pm 0.63 \text{mm}^3$ ,  $p<0.001$ ). Ganglion cell layer (GCL) thickness in the perifoveolar area in glaucoma eyes was significantly below normal and averaged  $34.4 \pm 9.65 \mu\text{m}$ , the thickness of the temporal section was lower than the nasal ( $34.20 \pm 9.53 \mu\text{m}$ ,  $39.06 \pm 10.60 \mu\text{m}$ , resp.). GCL is affected earlier than the changes in other layers of macular area.

**Conclusion** The distinctive feature of glaucomatous optic neuropathy in CG is a combination of significant structural changes in the optic nerve and retinal layers due to secondary distension of the membranes of the eye, with retinal vessels involved in the process, with some hemodynamic changes in the retina and choroid, all of which worsened with the progression of glaucoma.

**Poster session 2 : Physiology-Biochemistry-Pharmacology / Retina-Vitreous / Neuro-ophthalmology-Strabismology-Paediatric Ophthalmology-History of Ophthalmology**

• F093

**First outcomes of silicon rod frontalis suspension prospective follow-up in congenital blepharoptosis**

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**Purpose** Frontalis suspension is an efficient surgical solution for moderate to severe congenital blepharoptosis. Compared to autologous fascia lata, silicon rod allows faster surgery but long-term results are still needed. Aim: to assess the long-term results of silicon rod frontalis suspension

**Methods** This one year bicentric non-comparative prospective study included ten patients who underwent frontalis suspension using silicon rod sling (FCI, France). The main criterion was the palpebral height measured before surgery and at one week, three months and one year postoperatively. Palpebral height was measured with Image J using a standardized technique on digital picture

**Results** The intermediate results are shown. The palpebral height was significantly improved in all patients at one week and three months compared to the preoperative values but tended to decrease between one week and 3 months

**Conclusion** The intermediate results show that silicon rod frontalis suspension seems an efficient method to treat congenital blepharoptosis. One-year results are necessary to validate this outcome

• F094

**The Leonardo Da Vinci Program in the ophthalmological formation of residents**

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**Purpose** To present an EU sponsored program in the formation of residents in ophthalmology. With the introduction of the FEBO (federal board of education of ophthalmology) the formation of residents in ophthalmology in Europe has taken a big step towards equal standards. Nevertheless, differences still exist mostly due to different durations of residency programs, different priorities and different patient and disease spectrum in each country and each region of the EU.

**Methods** The Leonardo Da Vinci Program is designed as an educative program of lifelong learning and teaching. Three different groups can apply: 1. people during their formation 2. regular working people 3. persons working in the educational field Application can be performed online

**Results** Two persons were sent to United Kingdom (UK) and Spain (ES), with different objectives Basic investigation: one subject was able to obtain a profound insight into basic investigation at Nuffield Department of Clinical Neurosciences, UK. As a result of his stay many international collaborations were founded. Clinical routine: The second person was able to obtain an insight into the clinical routine at the Hospital Nuestra Señora de la Candelaria, ES. Especially surgery of ptosis and the keratitis due to acanthamoeba were strong points in the clinical routine. As a result a vivid exchange between hospitals was established.

**Conclusion** Regional differences concerning clinical routine and basic investigation in the EU will always exist due to different circumstances in different countries. Residents will profit in their personal and professional formation taking advantage of the existing EU-scholarships and thus improving patient care on a European level.

• S001

**Mesenchymal stem cells as an alternative source of stem cells for ocular surface regeneration**

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**Purpose** To test the therapeutic potential of mesenchymal stem cells (MSCs) to replace limbal stem cells (LSCs) for the reconstruction of the heavily damaged ocular surface in an experimental mouse model.

**Methods** MSCs were isolated from the bone marrow of BALB/c mice and expanded in vitro. Their phenotypic markers, differentiation potential, immunoregulatory properties and ability to transdifferentiate into corneal epithelial cells were characterized. These in vitro expanded MSCs were transferred, using nanofiber scaffolds, onto the damaged ocular surface and their therapeutic potential was evaluated.

**Results** MSCs can be expanded in vitro and retain their differentiation potential. When they were cultured in the presence of an extract from a damaged cornea or in the supernatant from cultured corneal cells, MSCs expressed cytokeratin CK12, a marker of corneal epithelial cells. In addition, MSCs displayed strong immunosuppressive properties in vitro and in vivo. After their transfer onto the damaged ocular surface, they supported corneal epithelial healing and inhibited the local inflammatory reaction. The therapeutic potential of MSCs was proven by the rapid regeneration of the ocular surface in the presence of MSCs and by an attenuation of the expression of proinflammatory cytokine genes after the injury.

**Conclusion** MSCs, which can be obtained relatively easily from bone marrow or adipose tissue, have the potential to replace LSCs for the reconstruction of the heavily damaged ocular surface.

• S002

**Augmented dried versus cryopreserved amniotic membrane as an ocular surface dressing**

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**Purpose** Dried amniotic membrane (AM) can be a useful therapeutic adjunct in ophthalmic surgery and possesses logistical advantages over cryopreserved AM. Differences in preservation can significantly influence the bio-chemical and physical properties of AM, affecting clinical efficacy. This study investigates a novel drying technique and the biochemical and structural effects on AM and following pre-treatment of AM with novel lyoprotectants.

**Methods** AM was cryopreserved (CPAM) or dried (DAM) with and without pre-treatment with lyoprotectants. Structural and visual comparisons were assessed using electron microscopy. Localisation, expression and release of AM biological factors were determined using immunofluorescence and immunoassays. The biocompatibility of AM preparations co-cultured with primary corneal epithelial cell (CEC) or keratocyte monolayers were assessed using standard cellular health assays.

**Results** Drying devitalised AM epithelium, but less so than cryopreservation, and cellular damage was reduced in DAM pre-treated with lyoprotectant. DAM alone, and pre-treated with lyoprotectant showed greater factor retention efficiencies and bioavailability compared to CPAM. In addition cellular health assays showed that DAM alone and pre-treated with lyoprotectant are compatible and superior substrates compared to CPAM for CEC expansion, with increased proliferation and reduced cytotoxicity profiles. This was supported by improved wound healing in CEC co-cultured with DAM and pre-treated DAM, compared to CPAM.

**Conclusion** Our modified preservation process and our resultant optimised DAM has enhanced structural properties and biochemical stability and is a superior substrate to conventional CPAM.

• S003

**High doses of fatty acids for treating severe dry eye disease**

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**Purpose** To evaluate the effect of high doses EPA/DHA for the treatment of severe dry eye disease not responding to current treatments.

**Methods** 60 eyes (30 patients) with severe dry eyes not responding to current treatments were treated with 5g/day of EPA/DHA. Visual acuity, corneal fluorescein staining, tear break up time (TBUT) and subjective symptoms were noted.

**Results** 70% of patients used more than 5 treatment approaches before starting EPA/DHA. 83% of patients started with severe corneal fluorescein staining and 17% had moderate staining. At 3 months 44% had no staining and 55% had mild staining. TBUT improved from mean 1.78sec to 4.40sec at 3 months. There was substantial improvement (>50%) on subjective symptoms at 6 weeks of treatment.

**Conclusion** High doses of fatty acids can be used to improve the signs and symptoms of patients with severe dry eye disease not responding to current treatments.

• S004

**Tear secretion impairment as a function of severity of herpetic keratitis**

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**Purpose** To assess the quality of tear secretion in eyes of patients with a history of unilateral and recurrent herpetic keratitis

**Methods** 33 patients with a history of recurrent herpetic keratitis (either archipelago keratitis, KA, or kerato-uveitis, KU, or neurotrophic keratitis, KN) were compared with 33 normal subjects. A complete ophthalmologic examination was performed, with successively the assessment of tear osmolarimetry, tears break-up time (TBUT), Schirmer I test, and corneal sensitivity. Patients with other potential causes of abnormal tears were excluded, and all tests were performed at least 3 months after the last relapse of keratitis. Controls were selected among asymptomatic patients scheduled for cataract surgery or refraction disorders, and were matched for age and gender with patients.

**Results** The patients group (19 men, 14 women, aged  $52 \pm 7$  years) included 16 patients with KA, 13 with KU, and 6 with KN. In the control group (similar sex ratio and mean age), all tests were symmetrical between the two eyes. In the 3 groups of patients, tear osmolarimetry was significantly greater in affected eyes than in controls, as well as TBUT was significantly reduced. In contrast, Schirmer I test was reduced only in eyes of patients with a history of KU or KN. Finally, only KN eyes were statistically less sensitive than healthy eyes.

**Conclusion** Recurrent herpes keratitis induced changes in lacrimal secretion, even when the disease is apparently quiescent, and the abnormalities are more important as the corneal disease is progressing. In the context of our study, tear hyperosmolarity appeared to be a particularly sensitive test to detect impairment of tear secretion.

• S005

**Corneal nerve structure and function in patients with non-Sjögren dry eye**

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**Purpose** To evaluate the relationship between the in vivo morphology of subbasal corneal nerves and clinical parameters in patients with non-Sjögren dry eye disease (NSDD).

**Methods** Forty-three patients with NSDD and 14 healthy age- and gender-matched control subjects were included. Each patient underwent an evaluation of ocular surface disease symptoms using the Ocular Surface Disease Index (OSDI), tear film break-up time (TBUT), corneal and conjunctival staining (Oxford scale), the Schirmer test, corneal sensation testing using the Cochet-Bonnet esthesiometer and corneal subbasal nerve analysis with IVCM. One eye of each subject was included in the study.

**Results** Corneal sensitivity was significantly lower in the dry eye group as compared to the control group ( $P=0.001$ ). Corneal subbasal nerves showed significant changes in dry eye patients as compared to normal subjects: lower density ( $P<0.001$ ), increased tortuosity ( $P<0.001$ ), number of beadings ( $P<0.001$ ) and width ( $P=0.014$ ). In patients with dry eye, corneal subbasal nerve density was correlated to age ( $r=-0.335$ ,  $P=0.028$ ), the Oxford scale ( $r=-0.562$ ,  $P<0.001$ ) and central corneal sensitivity ( $r=0.305$ ,  $P=0.047$ ). The maximum length of nerve fibers within a frame (MaxL) was correlated to the OSDI ( $r=-0.332$ ,  $P=0.030$ ) and the Oxford scale ( $r=-0.321$ ,  $P=0.036$ ). In multivariate analysis corneal subbasal nerve density remained correlated to the Oxford score ( $P<0.001$ ). The Oxford score was also correlated to central corneal sensitivity ( $r=-0.317$ ,  $P=0.039$ ).

**Conclusion** NSDD patients presented significant alterations of subbasal corneal nerve structure and function. In NSDD, subbasal nerve alterations were correlated to ocular surface tissue damage.

• S007

**Effects of cyclosporine in an experimental rat model of dry eye**

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**Purpose** Dry eye syndrome is a relatively common disease with multifactorial causes. It has been shown that rodent models of dry eye experimentally induced by scopolamine, a tropane alkaloid drug with muscarinic antagonist effects, could be helpful to test and select therapeutic candidates in the disease. Here we propose to show the action of cyclosporine A, an inhibitor of T-cell activation and inflammatory cytokine production, after oral and topical administrations.

**Methods** Experimental dry eye was induced in female albino rats by a systemic and continuous delivery of scopolamine (20 mg/day) over 21 days via osmotic pumps implanted subcutaneously on day 1. Animals were divided in three groups of five animals: The first two groups were instilled either saline or cyclosporine 0.05% eye drops and the third group received 20mg/kg/day cyclosporine by oral administration. Tear production was measured with the phenol red thread test, tear break-up time was studied under slit-lamp, and corneal defects were examined by slit-lamp observation using blue light after 0.5% fluorescein eye drop instillation and in vivo confocal microscopy. These examinations were performed in both eyes at baseline and on days 7, 14 and 21.

**Results** Cyclosporine orally or topically administered significantly reduced clinical signs of dry eye by increasing lacrimation and decreasing corneal defect.

**Conclusion** Cyclosporine appears to show efficacy in this model, regardless of the mode of administration.

**Commercial interest**

• S006

**The usefulness of corneal confocal microscopy to assessing and monitoring changes associated with hematopoietic diseases**

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**Purpose** Evaluation of corneal confocal microscopy in vivo to assess and monitor changes in the course of hematopoietic proliferative diseases.

**Methods** In 3 patients (2 males and 1 female) examined because of suspicion of an unknown keratopathy we performed the following: basic slit lamp examination, corneal scans using Scheimpflug camera (Pentacam, Oculus) and corneal confocal microscopy in vivo (Rostock Cornea module, Heidelberg Engineering Retina tomograph III). All patients were sent to hematology consult.

**Results** On a slit lamp examination we described macroscopic, polymorphic deposits of crystals, located in the full thickness of corneal stroma. In the image from Scheimpflug camera - diffusive crystals causing increased hyperreflexive stroma. The corneal confocal microscopy images showed polymorphic crystalline deposits within the corneal stroma reaching the level of the endothelium from Bowman membrane. After exclusion of hypercholesterolemia and cystinosis, patients were referred to the hematology consultation, where the following hematopoietic diseases were diagnosed: multiple myeloma, monoclonal gammopathy, light chain disease.

**Conclusion** Corneal confocal microscopy is a useful tool for the detection and differentiation of the etiology of crystalline keratopathy. Performed at the appropriate stage can help in the early detection of disorders of the hematopoietic system.

• S008

**Ocular tolerability of preservative-free prostaglandin eye drops**

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**Purpose** Taflotan® (tafluprost) and Monoprost® (latanoprost) are preservative-free anti-glaucoma eye drops. In order to compare the ocular irritation potential these preservative-free prostaglandin products were instilled repeatedly on rabbit eyes. Cell viability in vitro was studied using immortalized human corneal epithelial (HCE) cells.

**Methods** To study the in vitro cytotoxicity of Taflotan and Monoprost, HCE cells were exposed to the test solutions for 5 to 30 minutes, after which the cytotoxicity was evaluated by MTS assay. Secondly, to compare their in vivo ocular tolerance, Taflotan or Monoprost solution was instilled 10 times at 30-minute intervals onto eyes of male albino rabbits. Left eyes were treated while right eyes remained as untreated controls. Besides scoring macroscopic findings using MacDonald-Shadduck system, slit lamp examination and corneal fluorescein staining were performed after the last instillations.

**Results** In MTS test, the 30 minutes treatment with Taflotan had no effect on the cell viability, whereas the cell viability was decreased by Monoprost treatment. After repeated instillations on rabbit eyes conjunctival redness and swelling were observed in both groups. Their irritation scores and occurring frequency were lower in Taflotan treated eyes than in those treated with Monoprost. In addition, discharge was observed only in Monoprost treated eyes from 30 minutes until 3 hours after the last instillation.

**Conclusion** Under the conditions of this study, the ocular irritation potential of Taflotan was weaker than that of Monoprost. The results indicate that Taflotan eye drops may have a better ocular tolerability profile than Monoprost.

**Commercial interest**

• S009

**Optimisation of a standardised chemical burn animal model for use in corneal wound healing**

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**Purpose** The rabbit ocular surface has been used for many years as a reliable comparison for the study of human corneal injuries. This work aimed to create a standardised corneal epithelial injury using an alkali insult, which results in a type III healing response with wound healing. The ultimate aim is the use of this in vivo injury model to test novel ocular bandages and substrate treatments.

**Methods** De-epithelialisation first required in vitro optimisation for reproducibility before use in *in vivo* rabbit models. Corneas obtained from New Zealand White rabbits were de-epithelialised with sodium hydroxide (NaOH) solution using a range of concentrations, durations and modes of application. The injury area, depth and severity were assessed using haematoxylin and eosin staining and transmission electron microscopy. The optimised in vitro model was applied to an *in vivo* pilot study in rabbits and tested for reproducibility using normal and fluorescein slit lamp images.

**Results** In vitro optimisation revealed that higher NaOH concentrations and extended durations caused undesirable irreversible damage to the cornea. Lower concentrations and durations produced the injury required. The injury model was established in an *in vivo* pilot study using our optimal parameters and tested to see if a chemical burn with little variation in area or severity could be achieved. Following 48 hour treatment we were able to detect reliable wound healing using our refined model.

**Conclusion** The *in vitro* optimisation showed us parameters that were sufficient to de-epithelialise the cornea, creating an injury without causing stromal damage. The model is now in *in vivo*, to test the biocompatibility and efficacy of various ocular surface substrate treatments.

• S010

**Treatment of Meibomian gland disease with the Lipiflow® system: A prospective study**

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**Purpose** To evaluate the safety and efficacy of Lipiflow® in the treatment of meibomian gland disease (MGD).

**Methods** Twenty eyes of 17 consecutive patients with MGD and evaporative dry eye were included in this study. MGD was associated with ocular rosacea in 58.8% of cases. There were 9 women (52.9%) and 8 men (47.1%) with a mean age of  $58.1 \pm 16.2$  years (28-82 years). Patients underwent a complete ophthalmologic evaluation including two symptoms questionnaires, SPEED® and ocular surface disease index (OSDI), tear film break-up time (TBUT), Schirmer test, meibomian gland evaluation with the Meibomian Gland Evaluator® (MGE), tear film thickness measured with the Lipiview® Ocular Surface Interferometer and tear osmolarity (Tearlab®). Then all eyes were treated with the Lipiflow® system. All patients were evaluated before and one month after treatment.

**Results** According to SPEED questionnaire, symptoms improved significantly one month after treatment ( $15.4 \pm 4.9$  vs.  $12.7 \pm 5.8$ ; p<0.001). However, OSDI was not modified ( $22 \pm 8.4$  vs.  $21.7 \pm 9.5$ , p=0.06). After treatment, TBUT increased ( $3.1 \pm 1.6$  sec. vs.  $5.8 \pm 2.5$  sec., p<0.001) and the number of functional meibomian glands was significantly higher ( $2.8 \pm 1.1$  versus  $6.8 \pm 2.3$ , p<0.001). Schirmer test ( $13.4 \pm 12.2$  vs.  $15.1 \pm 14$ , p=0.6) and tear film thickness ( $57.3 \pm 21.8 \mu\text{m}$  vs.  $62.5 \pm 23 \mu\text{m}$ , p=0.15) were not modified after treatment. Similarly, no change was observed in tear osmolarity ( $321.3 \pm 19.1 \text{ mOsm/mL}$  versus  $321.7 \pm 24.1 \text{ mOsm/mL}$ , p=0.79). No adverse effect was observed for any patient during treatment and follow-up.

**Conclusion** The use of the Lipiflow® system was safe and seemed to improve symptoms and some ocular surface parameters in eyes with MGD.

• S011

**Impact of two different prostaglandin preparations on human corneal epithelial cells (HCE-2) in *in vitro* conditions**

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**Purpose** To investigate impact of two antiglaucoma prostaglandin preparations on human corneal epithelial cells in *in vitro* conditions.

**Methods** Human corneal epithelial cells (HCE-2), cultured in standard conditions, were treated with two commercially available preparations of prostaglandins: latanoprost and tafluprost. Treatment was applied in two time points – parallel with cells plating (day 0) or after 48 hours from plating (day 2). Medium containing drugs with % concentration of 0.1, 0.3, 1, 3, 10 was changed every second day. As negative control standard medium was used, as positive – medium containing 3% methanol. After 48 hours of culture, living culture was stained with erythrosine to visualize dead cells. After fixation, immunostaining for Ki67 antigen and ubiquitin (proteasomal marker) was performed.

**Results** In latanoprost group "day 0", concentrations <1% affected cells attaching and proliferation rate, >1% made cells totally unable to attach. In tafluprost group "day 0", concentrations <3% have shown no visible effect on cells, for concentrations >3%, fluctuations in cell shape appeared. In group "day 2" latanoprost treatment caused massive cells detachment for concentrations >1%. For lower concentration induced decrease of cells proliferation. Tafluprost treatment in this group showed no visible changes comparing with negative control, except 10% concentration, where induced mild cells detachment. In both groups treatment decreased expression of Ki67 and increased cellular ubiquitin (much more expressed in latanoprost group).

**Conclusion** Latanoprost preparation revealed to be more harmful considering proliferation, proteolysis and toxicity for HCE-2 cells in comparison to tafluprost.

• S012

**Tear levels of loteprednol etabonate following instillation of a novel gel formulation**

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**Purpose** A new gel formulation of loteprednol etabonate (LE) has been developed which is non-settling, provides a pH close to physiological, and a reduced level of benzalkonium chloride. This study sought to examine the levels of LE in tear fluid following a single topical instillation of LE ophthalmic gel 0.5% to healthy volunteers.

**Methods** Single center, open label study. Twelve healthy subjects received LE ophthalmic gel 0.5% in one eye and tear sampling was performed at 6 hours (h), 9 h, 12 h, and 24 h after instillation. LE levels in tears were determined by LC-MS/MS. Ocular and non-ocular treatment emergent adverse events (AEs) were recorded.

**Results** 17 subjects were screened and 12 were enrolled. Mean age was 46 years and all but one subject was male. LE was detected in all tear fluid samples at all time points assessed. Mean (SD) LE levels were 114 (116)  $\mu\text{g/g}$  at 6 h, 19.7 (18.2)  $\mu\text{g/g}$  at 9 h, 26.9 (29.6)  $\mu\text{g/g}$  at 12 h and 2.41 (3.36)  $\mu\text{g/g}$  at 24 h. No drug-related AEs were reported.

**Conclusion** LE administered in the gel formulation remains present in tear fluid 24 hours after a single instillation. These results suggest a long residency time of LE on the ocular surface with the gel formulation. No safety concerns were identified.

*Commercial interest*

• S013

**In vivo confocal microscopy of cystic lesions in corneal disorders**

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**Purpose** The purpose of this study was to assess the correlation between slit-lamp biomicroscopy and in vivo confocal microscopy and to describe the morphology of corneal intraepithelial cystic lesions by in vivo confocal microscopy in several corneal disorders.

**Methods** 15 patients with clinically diagnosed Cogan Dystrophy (9), Meesmann's Dystrophy (2), Acanthamoeba Keratitis (2), Microsporidial Keratitis (1) and Incontinentia Pigmenti (1) were examined by slit-lamp biomicroscopy and in vivo confocal microscopy. Confocal microscopy images were used to assess size, shape and reflectivity of the corneal cystic structures.

**Results** Confocal microscopy was able to identify cystic lesions in all cases in which cysts were observed by slit-lamp biomicroscopy. In vivo confocal scans showed multiple brightly reflective round, oval or circular structures with diameter from 10 microns (Incontinentia Pigmenti) to 140 microns (Meesmann's Dystrophy). Large intraepithelial clefts were observed in Microsporidial Keratitis and Meesmann's Dystrophy.

**Conclusion** In vivo confocal microscopy provides a detailed microstructural image of cystic intraepithelial lesions in various corneal disorders and may play a role in appropriate diagnosis.

• S014

**Full-field optical coherence tomography of human donor and diseased corneas**

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**Purpose** To evaluate the performance of a full-field optical coherence tomography (FF-OCT) system in the study of human donor and diseased corneas and assess its suitability for use in eye banks

**Methods** Our study was carried out using a full-field OCT system from LLTech \*, developed for non-invasive imaging of tissue structures in depth. Images were acquired on human donor corneas (in normal and oedematous conditions) and surgical specimens of diseased corneas (Fuchs dystrophy, keratoconus, stromal scar after keratitis)

**Results** The Full-field OCT device from LLTech\* enabled three dimensional images to be obtained with ultrahigh resolution (1 micron in all directions) comparable to traditional histological sections. This allowed a precise visualisation of the cells and the different structures (epithelium, Bowman's membrane, stroma, Descemet's membrane and endothelium) in normal corneas, but also in diseased corneas (even in the presence of an oedema), with specific lesions in each condition.

**Conclusion** Optical and specular microscopy, with a detailed view of the corneal endothelium and a cell density determination, remain the « gold standard » in the study of human cornea grafts. However, full-field OCT, thanks to a more complete anatomical study of the cornea, could be helpful in the evaluation and the selection of human cornea grafts which quality plays a major role in the outlook of the corneal transplant

• S015

**Aquaporin expression in pterygium**

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**Purpose** Pterygium is an ocular surface disease associated with epithelial and fibrovascular outgrowth from the conjunctiva onto the cornea. Aquaporins are transmembrane water channels proteins implicated in cell migration and proliferation. This work aimed at analyzing the expression of AQP1, AQP3 and AQP5 in pterygia samples, compared to normal conjunctiva tissue.

**Methods** Twenty five surgically removed pterygia were used for this study. Normal conjunctiva was obtained from 7 human cadaveric donors. The expression of AQP1, AQP3 and AQP5 was measured by real-time quantitative PCR (qRT-PCR). The cellular distribution of those AQPs was assessed by immunohistochemistry.

**Results** The expression of AQP1, AQP3 and AQP5 mRNAs was detected by qRT-PCR in pterygia. In normal conjunctiva and pterygia, AQP1 was expressed on endothelial cells, myofibroblasts (but not on fibroblasts) and some inflammatory cells. AQP3 and AQP5 were expressed on epithelial cells and inflammatory cells. AQP5 was also expressed on fibroblasts and myofibroblasts. In pterygia, the epithelial expression of AQP3 and AQP5 was decreased at the leading edge of the lesion as compared to normal conjunctiva. AQP1 was strongly expressed in myofibroblasts in fibrotic lesions of pterygia.

**Conclusion** AQP1, AQP3 and AQP5 are expressed in pterygia. Variation of AQP3/AQP5 epithelial expression and the strong induction of AQP1 expression in myofibroblasts suggest that those AQPs might be implicated in the pathogenesis of pterygia.

• S016

**Expression of Huntingtons disease markers in the cornea of minipig transgenic for the human mutated huntingtin**

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**Purpose** Huntingtons disease (HD) is a neurodegenerative disorder that is caused by an expansion of a polyglutamine (polyQ) domain in the protein of huntingtin (htt). PolyQ expansion above 35–40 results in disease associated with htt aggregation into inclusion bodies. The recent studies have shown that the illness arises not only in neurons, but also in non-neuronal cells. Purpose of this study was to investigate the expression of selected HD markers (on htt and their aggregates, ferritin and ubiquitin) in corneas of minipig transgenic for the human mutated huntingtin and compare them with corneas of wild type (WT) minipig.

**Methods** In this study we used WT minipigs and minipigs transgenic for N-terminal part of the human mutated huntingtin (both F2 generation, age 24 months). HD markers were examined on cryostat sections immunohistochemically by using the following primary antibodies: anti-polyglutamines, anti-polyglutamine-expansion diseases marker, MW8 marker, anti-ferritin and anti-ubiquitin.

**Results** Immunohistochemical examination on huntingtin demonstrated the increased expression of this protein in corneal epithelium and keratocytes of transgenic minipig in comparison with wild type cornea. The expression of ferritin detected in epithelial cells was very low in both studied corneas without significant difference between them. No nuclear inclusions or ubiquitous proteins were found in the cornea of transgenic minipig.

**Conclusion** This study showed that preclinical (silent) stages of HD are characterized by an elevated level of mutagenic huntingtin without formation of nuclear inclusions and ubiquitination of proteins. Supported by CHDI Foundation (A-5378 and A-5379), TA01011466, CZ.1.05./2.1.00/03.0124 and RVO: 67985904.

• S017 / 3676

**Various epithelial manifestation of herpetic keratitis in corneal confocal microscopy imaging**

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**Purpose** To present different variants of epithelial changes observed in patients with herpetic keratitis in corneal confocal microscopy *in vivo* images.

**Methods** In 19 patients with diagnosed herpetic keratitis of typical clinical course, we performed the following: basic slit lamp examination, corneal scans using Scheimpflug camera and corneal confocal microscopy *in vivo*. Full thickness corneal scans were made and compared with corresponding images from healthy objects. Additional imaging of epithelial layer with higher magnification was made to compare detailed changes within all components of epithelial layer.

**Results** On a slit lamp examination we described different size and surface ulceration of typical dendritic shape. In the image from Scheimpflug camera – local turbidity of stroma and lack of superficial layers in place of ulceration. The corneal confocal microscopy images showed different severity of stromal changes in form of hazing, turbidity, keratocytes activation, inflammatory cells infiltration or fibrosis. In epithelial layer scans except mostly diffused inflammatory infiltration, we described changes involving: epithelial cells-intracellular inclusions, abnormal cells shape and size, degeneration; intercellular matrix-hazing, inclusions; superficial nerve plexus-thinning or lack of nerves, mace expand of nerves, nerve fibers proliferation; Bowman membrane and anterior stroma-epithelial hernias into deeper layers.

**Conclusion** Herpetic corneal inflammations have no typical image in corneal confocal microscopy; however can show repeatable features in epithelial layer, which are helpful in diagnosis. Corneal confocal microscopy is useful tool for recognizing viral keratitis.

• S018

**Ocular surface dysfunction and tear osmolarity after cataract surgery**

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**Purpose** The aim of the study was to evaluate subjective symptoms, tear film break up time (BUT) and tear osmolarity before the cataract surgery and in the early postoperative period.

**Methods** 32 patients undergoing cataract surgery were included into the study. They were submitted to clinical evaluations including the administration of Ocular Surface Disease Index (OSDI) questionnaire, visual acuity, slit lamp examination, estimation of BUT. Tear samples were collected for osmolarity evaluation. All the tests were performed day before surgery, one week and one month after surgery.

**Results** Before the surgery 11 (47.8%) patients had no or mild dry eye disease, 21 (52.2%) – moderate and severe. The mean BUT was 7.4±3.2s and decreased in one week and one month after surgery (5.13±2.54s); paired differences were significant. Tear osmolarity was 306.25±15.38 mOsm/l with tendency to increase in one week (307.32±17.13) and decreased one month after surgery (293.7±24.05); paired differences were significant. OSDI score had tendency to increase one week and month after the surgery.

**Conclusion** Patients complaints increased in early postoperative period. Tear film stability significantly decreased one week and one month after surgery with the significant decrease of tear osmolarity. Decreased tear film stability could influence ocular discomfort after cataract surgery.

• S019

**Ultrastructure features of dhuh lizard (*Uromastyx aegyptia*) cornea**

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**Purpose** The Dhuh lizard (*Uromastyx aegyptia*) belongs to the family Agamidae and sub-family Uromasticinae, which is native of very dry and harsh desert climates. We report ultrastructural features of the dhuh lizard cornea.

**Methods** Four corneas of dhuh lizards were fixed in 2.5% glutaraldehyde containing cuproline blue in sodium acetate and magnesium chloride buffer. The tissue was washed in the buffer and dehydrated in a graded series of ethanol. The tissue was embedded in spur resin. The ultrathin sections were observed under a JEOL 1400 transmission electron microscope.

**Results** The cornea of the dhuh lizard is very thin (approximately 28 $\mu$ m) and consists of 4 layers: epithelium, stroma, Descemet's membrane and endothelium. The sub-epithelial lamellae of the anterior stroma consisted of three or four collagen fibrils interlaced with each other. The lamellae in the anterior, middle and posterior were also thin and running parallel to each other. The epithelium constitutes approximately 14% of the cornea, whereas the stroma constitutes 80% of the cornea. The mean diameter of CF and PGs mean area in the anterior stroma were larger compared to the mean diameter and mean PGs area of the middle and posterior stroma.

**Conclusion** The relatively thick epithelium of the cornea protects the stroma from drying out in the desert environment. The presence of large PGs suggests that they keep the hydration of the stroma at optimum level in the dry weather.

• S020

**Variability of oxidative stress-related genes in keratoconus and Fuchs endothelial corneal dystrophy**

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**Purpose** In the present work we investigated polymorphism in nuclear genes encoding proteins important for the cellular reaction to oxidative stress and mutations in mitochondrial DNA in two eye diseases with established role of oxidative stress in their pathology: keratoconus (KC) and Fuchs endothelial corneal dystrophy (FECD).

**Methods** Over 200 FECD and KC patients as well as more than 300 sex- and age-matched controls were enrolled in this study. Genetic variation in nuclear DNA was determined in peripheral blood lymphocytes by allele-specific and restriction length fragment polymorphism PCR. Quantitative real-time PCR with TaqMan probes was employed to study mutagenesis in mitochondrial DNA (mtDNA).

**Results** Genotypes of polymorphisms of genes encoding catalase and superoxide dismutase, glutathione transferase, enzymes neutralizing reactive oxygen species, were associated with the increased risk of AMD, FECD and KC. Polymorphism of numerous DNA repair genes, including HOGG1, MUTYH, UNG and SMUG1 was associated with AMD and KC. Finally, we found some associations between the occurrence of FECD and mtDNA mutagenesis, expressed by the number of mtDNA copies and specific deletions and point mutations, including 4977 bp deletion in mtDNA encoding 5 tRNAs and 7 proteins of respiratory chain as well as the 414T>G transversion.

**Conclusion** Polymorphism in nuclear genes encoding oxidative stress-related proteins and mutations in mitochondrial DNA may contribute to the risk of occurrence and development of KC and FECD by modulating the cellular reaction to the stress.

## • S021

**Galilei Dual Scheimpflug Analyzer: Corneal thickness and anterior chamber characteristics in healthy human eyes**

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**Purpose** To evaluate the repeatability of Corneal Thickness (CT) measurements and the normal values of Anterior Chamber Volume (ACV), iridocorneal angles and Anterior Chamber Depth (ACD) in healthy human eyes.

**Methods** Pachymetry and Anterior Chamber (AC) parameters were recorded prospectively in a consecutive case series of 52 normal eyes. All the studied subjects had no ocular abnormalities rather than mild refractive error less than D EE. Measurements were performed using the Galilei Dual Scheimpflug Analyzer.

**Results** There was a high intra-observer agreement, finding the best value in the thinnest central CT with an intra-class correlation coefficient (ICC) of 0.999 and the worst, in the peripheral CT, with an ICC of 0.807. ICC of 0.997 and 0.994 were found in central and paracentral CT respectively. The mean values  $\pm$  SD for the AC parameters were: ACD 3.29mm  $\pm$  0.27mm; ACV 123 mm<sup>3</sup>  $\pm$  49.05mm<sup>3</sup>; Temporal Angle ACD 37.58°  $\pm$  2.83°; Superior Angle ACD 36.71°  $\pm$  4.05°; Nasal Angle ACD 37.17°  $\pm$  3.19° and Inferior Angle ACD 38.55°  $\pm$  3.71°.

**Conclusion** Galilei device allows a noninvasive measurement of different AC parameters. These data provides benchmark information that can be used to monitor and assess clinical practice; besides pachymetry is found to be a highly repeatable parameter.

## • S022

**Pragmatic approach in a case of unknown bilateral corneal opacity and staphyloma in a child in Timor Leste**

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**Purpose** To demonstrate limited diagnostic and therapeutic options in ophthalmology in Timor Leste on the basis of a clinical picture of unknown corneal opacity.

**Methods** Clinical-pathological case report.

**Results** A six year old girl was referred into the clinic because of a progressive corneal opacity in both eyes - left more than right - known since 18 life months. Clinically a light perception of the right eye could be observed. The left eye was amaurotic. A complete grayish-white opacification of both cornea which impeded any clinical examination of anterior and posterior segment were found. The surface of the left cornea was obviously bulged out, accordingly a proper lid closure was impossible. Further diagnostic testings like ultrasound, CT-scanner, MRI or blood examination were not possible due to the limited resources. Assuming for a severe pathology and particularly a possible untraeted superinfection later on a evisceration was performed in general anaesthesia on the left eye. Histology done in Switzerland showed congenital corneal staphyloma with marked thickening of the cornea due to abnormal stromal connective tissue. Additionally, absence of Bowman and Descemet membranes as well as large anterior and focal posterior synechias of the iris were found.

**Conclusion** The present pathologic findings were compatible with a very rare manifestation of Peters anomaly combined with abnormally thick intracorneal fibrosis and congenital corneal staphyloma. In the absence of any clinical supplementary examinations, a malignant intraocular tumour could be excluded histologically.

## • S023

**Semiology of patient derivated to pterygium surgery at Fuan Noe c. Hospital in Arica city, Chile**

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**Purpose** Knowing the semiological characteristics of surgical patient diagnosed with symptomatic pterygium, who has permanent resident at the desertical city of Arica, Chile.

**Methods** A transversal type study of 119 people was performed by applying them a survey, a physical exam and a pre surgical ophthalmologic evaluation. After surgery the removed tissue was sent to anatomopathological analysis. The survey data and test results were tabulated and analyzed using the statistical software Stata 11.0

**Results** Over 90% have permanent residence for more than 11 years in this desertical region; 89% work outdoors, 87.1% have nasal pterygium, 54% reported never used sunglasses. 73% has another visual disturbance. The 45.16% presents Pterygium of angiomatic and 45.15% Mixed type. Only 9.68% is fibrous. A 18.28% present metaplasia. Over 60% presents altered the Schirmer test results (higher than 24mm) and 50% in Break Up Time (BUT) altered lower or equal to 10 seconds.

**Conclusion** The Pterygium is a disease with high prevalence in the desertical city of Arica, Chile and it is evident that the absence of laws in prophylactic measures and educational activities, increase the damage that is produced by ultraviolet radiation. The relationship between the habitability of the desert and the correspondence with the use or not of lenses with UV protection is proportional.

## • S024

**Evaluation of transplanted oral epithelium by confocal microscopy correlates with success rate in aniridia patients**

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**Purpose** To evaluate success of transplanted oral epithelium in corneal surface reconstruction in aniridia patients

**Methods** 25 patients suffered from congenital aniridia accompanied by aniridial keratopathy underwent COMET procedure. Confocal microscopy examination was performed 7, 14 and 28 days aftertransplantation. Number of cell layers, cell density and stability of epithelium was evaluated

**Results** The survival of transplanted oral epithelium is corelated with early postoperative evaluation of epithelial graft

**Conclusion** Confocal microscopy examination could be useful to predict survival time of oral epithelialgraft

• S025

**Effects of acute in vivo exposure of rabbit cornea and conjunctiva to artificial sunlight**

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**Purpose** Acute exposure of ocular and periocular tissues to natural or artificial light can induce inflammatory responses, attributed mainly to the UVB range of the spectrum. In this study, we have used a model of acute exposure of rabbit eyes to artificial sunlight, to study possible alterations in the architecture of corneal and conjunctival tissue as well as in the expression of tumor necrosis factor (TNF) and platelet activating factor (PAFR) in these tissues.

**Methods** New Zealand albino rabbits were immobilized opposite a 300W light bulb and exposed for 30 min to an equivalent of 7,500 Jm<sup>-1</sup> of UVB irradiation, in the range of the reported threshold for corneal damage. Corneal and conjunctival tissue samples were removed from exposed eyes at 2, 6 and 24 hours following the end of the exposure to the bulb light, and were subsequently processed for histochemical staining or RNA extraction. The gene expression of TNF and PAFR was monitored with RT-PCR.

**Results** Histopathological examination revealed minor changes in the corneal architecture. A distinctive eosinophilic infiltration was observed, as early as 2 hrs post-exposure, in the conjunctiva of the third eyelid, both in the epithelial layer and the basal lamina, which had apparently subsided by t = 24 hrs. The gene expression of TNF and PAFR was monitored with RT-PCR.

**Conclusion** Acute exposure to artificial sunlight caused a marked accumulation of eosinophils in rabbit conjunctival epithelium but only minor changes in the corneal epithelium. Neither TNF nor PAFR gene expression was affected appreciably

• S027

**Management of HSV-1 necrotizing keratitis with amniotic membrane transplantation combined with antiviral and steroid therapy**

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**Purpose** To report promoted healing of necrotizing herpetic keratitis after amniotic membrane transplantation (AMT) in conjunction with antiviral and corticosteroid therapy.

**Methods** Herpes necrotizing stromal keratitis was defined as a corneal ulcer with a dense, whitish inflammatory infiltration and corneal scarring positive for HSV-1 by polymerase chain reaction (PCR). Corneal smears and cultures were made to rule out bacterial and fungal infections. All patients received systemic antiviral treatment and topical antibiotics. When corneal ulcers were refractory to medical treatment, multilayered AMT was considered. During the post operative period, systemic antiviral treatment was continued and local corticosteroids were started. Main outcome measures were visual acuity and wound healing of corneal ulcers.

**Results** Thirteen patients (13 eyes) with herpes necrotizing stromal keratitis were retrospectively reviewed. The mean follow up was 13.1 months (range 2-30 months). Amniotic membrane transplantation was performed in 9 cases (twice in one patient), within a mean of 17 days (range 2-43 days) after medical treatment initiation. Epithelial defects healed within a mean of 40 days (range 9-97 days) after AMT. At the last visit, mean visual acuity improved from 2.2 logMAR to 1.3 LogMAR (p=0.016). No serious side effects occurred during the follow up.

**Conclusion** Multilayer AMT combined with antiviral and corticosteroid therapy seems to be effective in treating herpes necrotizing stromal keratitis. It provides restoration of ocular surface integrity and improvement of vision.

• S026

**Prevalence of allergic conjunctivitis in allergic population in northern greece**

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**Purpose** A retrospective study on the prevalence of conjunctivitis and the commonest allergens in Northern Greece.

**Methods** From the archives of an outpatient unit in Thessaloniki-Greece, the patients with conjunctivitis were evaluated. The patients underwent skin prick tests (SPTs) according to current guidelines. The patients were divided into 4 groups: Group A : Patients with conjunctivitis alone, Group B: patients with asthma and conjunctivitis, Group C: patients with rhinitis and conjunctivitis, Group D: patients with all three co-morbidities.

**Results** 49 out of 1239 allergic patients had only conjunctivitis, 102 conjunctivitis and asthma, 117 conjunctivitis and rhinitis and 229 all three co-morbidities.

**Conclusion** In the Northern Greek population allergic conjunctivitis is common. There was no significant statistical difference between men and women in the aforementioned groups.

• S028

**Diagnosis of infectious keratitis by mass spectrometry**

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**Purpose** Microbial keratitis is an infection of the cornea that can be severe since it leads, in some cases, to blindness. One of the most important prognostic factors is the microbiological diagnosis which determines the rapid use of the appropriate treatment. Therefore we sought a faster and more efficient diagnosis method by using mass spectrometry.

**Methods** This study is the analysis of corneal samples with MALDI-TOF MS as a new approach, undeveloped in the literature, without preparation nor culture of infected epithelia. All together 18 human corneal epithelia were studied to characterize the healthy human corneal spectra. An animal experimentation was also conducted on 45 Balb/c mice for comparison of their left healthy corneas and their right infected corneas by two difficult diagnosis seeds: Mycobacterium chelonae or Acanthamoeba polyphaga.

**Results** Corneal protein spectra can be obtained quickly, in less than ten minutes, and easily by simple deposit on a mass-spectrometry target. The study of healthy corneas gives reproducible and specific spectra compared to other human tissues available in our database. In animal model, the MALDI-TOF MS detects an inflammatory response secondary to wound healing corneal and an infectious response to Mycobacterium chelonae.

**Conclusion** This original work consists in using MALDI-TOF MS for a microbial keratitis analyze directly on infected epithelium. This technique permits, to differentiate easily and quickly, a healthy cornea from an ulcerated or infected one in mice. After the development of microbiologic recognition algorithms, mass-spectrometry could be used, in the future, for a "minute" diagnosis of infectious keratitis. Diagnosis of infectious keratitis by mass spectrometry.

• S029

**Chitosan-based biocompatible materials in ophthalmology**

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**Purpose** The goal of current research was to develop the material for scaffold preparation providing optimal conditions for the corneal epithelium cell culturing and to determine their physicochemical and biological properties. The scaffolds, which could be applied in ophthalmology should fulfill some requirements such as biocompatibility, biodegradability, restorability, and also should have adequate mechanical strength

**Methods** A membrane was obtained from chitosan-polysaccharide derived from chitin. Chitosan is nontoxic, biologically inert, stable in the natural environment. It has found numerous applications in biomedical field, e.g. 3D scaffolds, fibers, membrane, hydrogels. These materials have shown support for adhesion, proliferation, expression of cells. The chitosan supports will be crosslinked with genipin, a product from the *Gardenia jasminoides*. The genipin is more than 5000 times less cytotoxic than typical chemical cross-linking reagents (glutaraldehyde). The mechanical properties of the supports obtained will be controlled mainly by the addition of other biopolymers

**Results** The in vitro cell culture experiments with corneal epithelium cells have indicated that a membrane prepared from chitosan blends provided the regular stratified growth of the epithelium cells, good surface covering and increased number of the cell layers. Some of chitosan membrane is comparable with that of the amniotic membrane which is currently recommended for clinical applications

**Conclusion** Novel polymeric hydrogel scaffolds for corneal epithelium cell culturing based on blends of chitosan with some other biopolymers such as hydroxypropylcellulose, collagen and elastin crosslinked with genipin, a natural substance, were prepared

• S030

**The use of tear osmolarity as a diagnostic tool for detection of dry eye prior to cataract surgery**

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**Purpose** To determine if the diagnosis of preoperative dry eye can help to improve outcomes following cataract surgery.

**Methods** This was a prospective study that enrolled cataract patients scheduled to undergo standard phacoemulsification surgery followed by placement of an intraocular lens. Patients were assessed for the presence of dry eye before surgery, and then at one week and one month after surgery. Tests used were tear break-up time (TBUT), Schirmer's, and tear osmolarity (TearLab Corp, San Diego, CA). All patients were placed on a standard postoperative treatment regime of topical steroids, non-steroidal anti-inflammatory drugs (NSAIDs) and antibiotics for one month following surgery.

**Results** Twenty-five patients were enrolled in the study. The mean preoperative tear osmolarity was 304.6 mOsm/L. Twelve patients had preoperative dry eye, defined as a tear osmolarity level greater than 308 mOsm/L. Mean osmolarity remained stable at the 1st postoperative visit. At the one-month postoperative visit, the mean osmolarity was 297.4 mOsm/L.

**Conclusion** The results suggest that conventional means of dry eye testing may miss patients with preoperative dry eye, compared to osmolarity testing, which provides an objective measurement. The lower postoperative osmolarity levels seen in this study were no doubt related to the postoperative medication regimen and it would be reasonable to expect that the osmolarity measurement would return to baseline after treatment stopped.

• S031

**Topical treatment with a new matrix therapy agent (RGTA) in combination with limbal allograft in ocular surface disease and corneal anesthesia**

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**Purpose** The surgical management of limbal deficiency is difficult, especially when it is bilateral and occupies more than half of the cornea. In these advanced cases, the presence of an associate corneal anesthesia, worsens the prognosis of allo-limbal transplantation. We aimed to assess the efficacy and tolerance of a new matrix therapy agent (RGTA, Cacicel20), mimicking heparan sulfates, in combination with limbal allograft for the reconstruction of the ocular surface with complete corneal anesthesia.

**Methods** It is a prospective, single-center study. Six eyes of six patients were included. All were men. The average age was 53 years [range : 35-87]. Mean follow-up was 4.8 months [range : 3-9 months]. Etiologies were corneal burn in three cases, an herpes infection in one case and idiopathic in 2 cases. All patients underwent limbal allograft associated with instillation of eye drops RGTA once every two days in the absence of healing after 7 days postoperatively. A topical steroid is prescribed in all cases. RGTA eye drops were stopped after complete healing. Patients were followed every 7 days until healing. Visual acuity, corneal sensitivity and the surface of the ulcer are evaluated each time using photography.

**Results** Despite the persistence of total corneal anesthesia, the epithelium healed in all cases before 2 months [range 7 days to 2 months]. Two of 6 patients showed significant improvement in their visual acuity. One patient had a corneal abscess regressing under treatment.

**Conclusion** RGTA seems to be a potentially useful, alternative, noninvasive therapeutic in association with limbal allograft in advanced limbal deficiency

• S032

**Simulation of DSAEK in a new corneal bioreactor**

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**Purpose** Penetrating keratoplasty or endothelial graft remain the only available treatments for several advanced corneal diseases. However there is an important mismatch between global needs and offer. Tissue bioengineering is booming and emerging as a promising solution to the current shortage of organs. Our laboratory is working on a large project of corneal bioengineering and has developed and patented a corneal bioreactor (BR) that allows restoration of transcorneal pressure gradient and continuous fluid circulation while allowing right through observation of the cornea with the main instruments available in ophthalmology. This BR will be next available for experimental works and for eye banking. Aim: to establish the proof of concept of integration and bio-functional evaluation of endothelial graft in the BR

**Methods** We performed a surgical simulation of DSAEK in our new corneal BR. Conventional Descemet stripping was performed and the denuded area covered with the endothelial lamella before placing the cornea into the BR. An air bubble was let in place for 2 hours and the pressure set at 30 mmHg and returned to 20 mmHg. Slit lamp examination, OCT with pachymetry and a study of transparency were made every 2 hours

**Results** Initial pachymetry was 1530 µm. After 24 hours, the corneal transparency improved and central corneal thickness dropped to 836 µm. No liquid interface between the endothelial lamella and the cornea was visible in OCT

**Conclusion** By reproducing physiological conditions, the BR allows simulation of a DSAEK. It represents an interesting tool for corneal bioengineering. Grant: ABM 2012, EFS 2012, ANSM 2012

• S033

**Risk factors for contact lens-related microbial keratitis: A case-control multicenter study**

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**Purpose** The most feared complication of contact lens (CL) wear is microbial keratitis (MK), even though its incidence remains low. While CL materials and replacement schedules have developed greatly in recent years, the risk of MK, however, has not been reduced in users of daily disposable and silicone hydrogel CL. This study aimed to identify the risk factors of CL-related MK while putting into perspective individual patients' risks and societal burdens.

**Methods** A multicenter case-control study was designed. The CL-related MK subpopulation (Case) was compared to healthy CL wearers (Control) using a complete a 52-item anonymous questionnaire designed to determine subject demographics and lens wear history. Univariate and multivariate logistic regression analyses were performed in order to compare both groups.

**Results** The study enrolled 508 cases and 499 controls, and revealed a higher risk for MK patients wearing soft CL, with cosmetic CL, with overnight wear, exceeding the CL- or the cleaning solution replacement delay, using a multipurpose cleaning solution. On the contrary, hyperopia, fitting and follow-up by an ophthalmologist, and respect of basic hygiene rules were protective against CL-related MK.

**Conclusion** The infectious determinants were linked to the patient, type of lenses, hygiene routine, CL handling, cleaning solution, and storage case. This study aimed to highlight the increasingly documented CL-associated complications, which likely occur due to insufficient health professional supervision and lack of patient information regarding basic rules of hygiene, as well as CL care and handling.

• S034

**Visual acuity outcomes after Descemet's stripping automated endothelial keratoplasty compared to penetrating keratoplasty**

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**Purpose** To evaluate the difference in visual recovery between Descemet's Stripping Automated Endothelial Keratoplasty (DSAEK) and Penetrating Keratoplasty (PK).

**Methods** Retrospective cohort study of 42 eyes. 21 eyes underwent PK compared to 21 eyes underwent DSAEK for a Fuchs' dystrophy or pseudophakic bullous keratopathy. Preoperative and postoperative best spectacle-corrected visual acuity (BSCVA) was recorded at 1, 3, 6 and 12 month. The PK and DSAEK procedures were performed by the same experienced surgeon with the same donor and similar recipient criteria. All patients underwent a standardized examination that included tonometry, visual acuity (distance and near) and biomicroscopic examination at 1 month, 3 month, 6 month and 12 month. Improvement in BSCVA between each point time was evaluated using paired-samples t tests.

**Results** The donor and recipient demographics were comparable in the PK and DSAEK groups. In the PK group, the visual recovery was significant after 12 month (1,71 logMAR +/- 0.33 to 1,25 logMAR +/- 0.58; P=0.023). The mean distance gain was 4,53 lines and the mean near gain was 3,89 lines. In the DSAEK group, the visual recovery was significant after 3 month (1,05 logMAR +/- 0,50 to 0,68 logMAR +/- 0,38; P=0,025). The mean distance gain was 4,25 lines and the mean near gain was 5,7 lines. The mean distance gain was equivalent between the two groups (P=0,91), and the mean near gain was statistically superior in the DSAEK group (P=0,47).

**Conclusion** There is a faster visual recovery and a more significant near gain after DSAEK surgery for Fuchs' endothelial dystrophy or pseudophakic bullous keratopathy.

• S035

**Uniformity of the thickness of LASIK corneal flaps made by IntraLase FS 60 laser femtosecond (IntraLase, AMO USA) measured by OCT spectral domain (Heidelberg, Germany)**

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**Purpose** The objective of this study was to appreciate the uniformity of the thickness of Lasik corneal flaps made by The IntraLase FS60 laser femtosecond (IntraLase, AMO USA).

**Methods** It was a retrospective, randomized, double-blind study.Twenty eight eyes were included in the study. Every flap was programmed to measure 120 µm. The flaps were measured by Spectral Domain OCT (Heidelberg), one month after surgery in five points on a horizontal line through the center of the pupil (center, 1mm and 2 mm from the center pupil in nasal and temporal)The scheduled and the obtained thicknesses were compared in each point.

**Results** Double reading by two different doctors showed no significant difference in the thickness flap measurements ( $p<0.3$ ).There was no significant difference between the thickness in each point of the flap and the center of the flap.

**Conclusion** We have shown in this study that the cutting of a corneal stromal flap with the IntraLase FS 60 laser femtosecond during a LASIK procedure realize uniform flap.

• S036

**Reproducibility of the thickness of Lasik corneal flaps made by IntraLase FS 60 laser femtosecond (IntraLase, AMO USA) measured by OCT spectral domain (Heidelberg, Germany)**

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Service d'ophtalmologie, Clamart

**Purpose** The objective of this study was to appreciate the reproducibility of the thickness of Lasik corneal flaps made by the IntraLase FS60 laser femtosecond (IntraLase, AMO USA).

**Methods** It was a retrospective, randomized, double-blind study.Twenty eight eyes were included in the study. Every flap was program to measure 120 µm. The flaps were measured by Spectral Domain OCT (Heidelberg), one month after surgery in five points on a horizontal line through the center of the pupil (center, 1mm and 2 mm from the center pupil in nasal and temporal)The scheduled and the obtained thicknesses were compared in each point.

**Results** Double reading by two different doctors showed no significant difference in the thickness flap measurements ( $p<0.3$ ).Average obtained thickness was 6, 84 µm greater than desired thickness (standard deviation of 7, 27 µm), ( $p<0, 01$ ).The average thickness flap was significantly greater: nasal 2mm: 128µm +/-7µm, nasal 1mm: 124,4µm +/-6µm, central: 127,7µm +/-8µm, temporal 1mm: 125,9µm +/-7µm, temporal 2mm: 128,2µm +/-8 µm ( $p<0,01$ ).

**Conclusion** We have shown in this study that the cutting of a corneal stromal flap during a LASIK procedure is significant thicker with the IntraLase FS60, but the standard deviation is low.

• S037

**Extracellular matrix derived hydrogel for corneal tissue engineering**

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**Purpose** Several different synthetic and natural hydrogels have been suggested for use in corneal tissue engineering however these hydrogels lack many of the matrix constituents present in the real cornea. To overcome this problem we developed a new hydrogel derived from decellularized porcine cornea extracellular matrix for use in corneal tissue engineering.

**Methods** Porcine corneas were decellularized using a combination of detergents and nucleases. The corneas were then freeze dried and milled into a fine powder. The powder was dissolved using a pepsin digest solution which underwent gelation at 37°C by neutralizing the solutions pH. Keratocytes were cultured on the hydrogels over several weeks to determine the hydrogels biocompatibility and ability to regulate cell behavior. Immunohistochemical staining was performed on the cell seeded hydrogels to examine the influence the hydrogel had on the cells phenotype.

**Results** Decellularization of the corneal matrix was confirmed using a DNA assay prior to matrix digestion. The hydrogels that were formed using this technique were highly transparent and able to maintain viable cells. Keratocytes seeded onto the hydrogels had fully infiltrated them after 2 weeks. Markers of keratocyte cell behavior were observed after 2 weeks in culture suggesting that the hydrogel supports the maintenance of a keratocyte phenotype.

**Conclusion** We have demonstrated a new hydrogel that can be used for engineering corneal tissue. The next step will be to test corneal epithelial with these hydrogels to determine their suitability for corneal transplantation.

• S038

**Age-related changes in central corneal thickness and corneal endothelial characteristics**

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**Purpose** To compare how corneal endothelial cell density, their average size and percentage of regular hexagonal cells depends on age; to estimate the average of endothelial cell density and central corneal thickness in different age groups.

**Methods** The data of 211 person (358 eyes), who were examined using a non-contact specular microscope at the Vilnius University Hospital Santariskiu Clinic Center of Eye Diseases, was analyzed. Parameters examined included corneal endothelial cell density, average size of cells, percentage of regular hexagonal cells, central corneal thickness. Patients age and sex were also noted. All subjects, whose age was from 20 to 89 years, were divided into 7 age groups, each involving 10 years.

**Results** A total of 114 (54.03%) subjects were women and 97 (45.97%) were men. A strong inverse correlation was observed between subject age and corneal endothelial cell density ( $r = -0.650$ ,  $p < 0.01$ ), weak inverse correlation between age and central corneal thickness ( $r = -0.156$ ,  $p < 0.01$ ) and weak direct correlation between cell density and central corneal thickness ( $r = 0.232$ ,  $p < 0.01$ ). Average size of cells was directly correlated with age ( $r = 0.586$ ,  $p < 0.01$ ). No correlation between age and percentage of regular hexagonal cells was found ( $p > 0.05$ ).

**Conclusion** Young individuals have higher endothelial cell density, which decreases with age. Corneal thickness also decreases, but its dependence on age is weaker. The lower cell density, the lower corneal thickness. Average size of cells is bigger in young subjects. Percentage of regular hexagonal cells does not depend on age.

• S039

**Differences in corneal epithelium in patients with diagnosed macular and granular corneal dystrophy**

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**Purpose** To describe severity and forms of epithelial changes in patients suffering from macular and granular corneal dystrophy.

**Methods** In examined group 9 patients were included, 4 with diagnosis of macular and 5 of granular corneal dystrophy. Patients underwent basic slit lamp examination and in next order in vivo corneal confocal microscopy. Full thickness corneal scans were made and compared with corresponding images from healthy objects. Additional imaging of epithelial layer with higher magnification was made to compare detailed changes within single cells.

**Results** On a slit lamp examination we described: in case of granular dystrophy macroscopic, polymorphic deposits of crystal structure, located in full thickness of corneal stroma and in epithelium. In macular dystrophy cornea stromal and epithelial changes took more diffused form. The corneal confocal microscopy images showed different intensity of typical stromal changes (crystalline or diffused depends on dystrophy type). In epithelium, in cases of macular dystrophy, we found different stages of intracellular changes, which secondary involved intercellular space. In granular dystrophy changes localized mostly in intercellular space with preserved cellular architectonics.

**Conclusion** However both described dystrophies are classified as stromal, in different way involve cells in epithelial layer. Corneal confocal microscopy can be useful for detailed examination of corneal dystrophic changes.

• S040

**Outcome of phacoemulsification after Descemet membrane endothelial keratoplasty (DMEK)**

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**Purpose** To evaluate the incidence of secondary cataract after Descemet membrane endothelial keratoplasty (DMEK), and the feasibility and outcome of phacoemulsification cataract surgery after DMEK.

**Methods** From a series of 106 consecutive phakic eyes that underwent DMEK for Fuchs endothelial dystrophy or bullous keratopathy, five (4.7%) required cataract surgery 9.2 ( $\pm 3.8$ ) months (range 4 to 14 months) after the initial DMEK. Outcomes after phacoemulsification were retrospectively assessed by reviewing the change in visual acuity, refractive error, endothelial cell density, pachymetry and the incidence of complications.

**Results** All phacoemulsification surgeries were uneventful and no dislocations and/or detachments of the Descemet graft were observed. At 6 to 12 months after phacoemulsification, all eyes reached a BCVA of  $\geq 20/30$  (0.6) and were within 0.5D of the intraocular lens power calculations. Endothelial cell density decreased from on average  $1535 (\pm 195)$  cells/mm<sup>2</sup> before, to  $1158 (\pm 250)$  cells/mm<sup>2</sup>. No significant changes in pachymetry values were observed up to 6 to 12 months postoperatively. All corneas remained clear throughout the study period.

**Conclusion** Phacoemulsification after DMEK can be performed with minimal risk of graft dislocation and may provide good refractive and visual outcomes with an acceptable decrease in endothelial cell density.

• S041

**New Image Plugin for rapid and reproducible measurement of viable corneal endothelial cell density**

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**Purpose** We previously described a laboratory test based on a triple Hoescht-Ethidium-CalceinAM labeling coupled with image analysis in order to determine the viability and mortality of endothelial cells on whole corneas (HEC-triple labeling, Pipparelli IOVS 2011). We then defined the new notion of viable endothelial cell density (vECD). Nevertheless the process comprised a manual linear thresholding liable to induce inter and intra-observer variability. Aim: to present a new ImageJ Plugin with improved image thresholding allowing rapid and reproducible vECD determination

**Methods** The endothelium of 10 human corneas unsuitable for graft was stained with HEC and its whole area observed with either a fluorescent microscope (IX81, Olympus), with motorized stage or a fluorescent macroscope (MVX10, Olympus). TIFF images acquired for H, E and C were imported in image J. Our new homemade plugin comprised contrast improvement, regional selection of pixels with similar gray levels, simplified thresholding of areas facilitated by a user-friendly display of images, possibility of manual touch up to increase accuracy. Repeated measurements of vECD were done by 5 observers to determine inter and intra rater variability, and compared to basic image thresholding

**Results** Inter and intra rater agreement was high. Compared to the basic thresholding, standard deviation of measurements was reduced by 66% ( $P=0.002$ , non parametric Wilcoxon ranked test)

**Conclusion** We made this new rapid and reproducible free image analysis tool available for the community to help standardize research works needing robust EC viability measurements

• S043

**Expression levels of purinergic receptors in the endothelium of human and murine cornea**

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**Purpose** Since loss of corneal endothelial cells (EC) due to cell death is the major problem in numerous corneal conditions as well as in tissue storage for corneal transplantation, finding ways to improve cell survival is of great importance. Purinergic receptors P2RY [G protein-coupled receptors] and P2RX [ligand-gated ion channels] are both known to be implicated in inflammation and apoptosis pathways, therefore modulation of these receptors may provide an efficient way to increase EC survival. The aim of this study was to study expression levels of P2RY2 and P2RX7 receptors (P2R) in murine and human corneal endothelium, as well as the association to the purinergic receptor ligand ATP.

**Methods** Expression levels of P2R were determined by qRealTime-PCR relying on untreated and Interleukin-1b-treated murine and human EC. EC apoptosis was also studied in response to ATP in a "cornea-in-a-cup" assay.

**Results** In murine EC P2RX7 were significantly predominant compared to P2RY1 and P2RY2, while in human EC P2RY2 were significantly prevailing compared to P2RX7. Exposure to ATP in increasing concentrations resulted in a steady increase of apoptosis. As indicators of EC stress, changes in cell size (polymegathism) and cell shape (loss of EC hexagonality, polymorphism) could be demonstrated.

**Conclusion** Our data describe distinct differences in P2R expression levels in murine versus human EC showing the limitations of using the mouse model in P2R EC research. As P2R seem to be evenly distributed over the entire concave surface of the EC monolayer, inhibiting P2R interaction with their ligands (e.g. extracellular ATP) may present an effective way to prevent EC apoptosis.

• S042

**Analysis of fungal contamination in contact lens storage cases between 2000 and 2012**

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**Purpose** The objective of this study was to identify fungi species isolated in contact lenses boxes from January 2000 to November 2012 and to compare with fungi species found in corneal samples over the same period.

**Methods** Retrospective study from January 2000 to November 2012. Over the same period, were analyzed isolates from 68 patients with clinical signs of keratomycosis confirmed by corneal samples culture. The temporal analysis (over the last 12 years) of the isolates was performed using the chi<sup>2</sup> test pattern.

**Results** 456/2433 contact lenses boxes fungi (18.7%). Non-filamentous fungi (yeasts) were isolated in 280 boxes (61.3%), including 85% of Candida (46% *Candida parapsilosis*) and 15% of other non-filamentous fungi. Filamentous fungi were isolated in 200 boxes (43.8%): *Fusarium* sp. (46.5%), *Penicillium* (23%), *Aspergillus* (12%) other filamentous fungi (18.5%). *Fusarium* sp. were isolated in 51.4% of patients with clinical signs of keratomycosis. A significant increase in filamentous fungi ( $\chi^2 = 6.4$  p <0.05) could be highlighted in contact lens boxes. Increase in *Fusarium* sp. in contact lens boxes is significant ( $\chi^2 = 17.5$  p <0.05) as the number of *Fusarium* sp. keratomycosis ( $\chi^2 = 6.2$  p <.05). Contact lens wear remains the main risk factor for keratomycosis (54% of patients).

**Conclusion** Increasing cases of *Fusarium* sp. keratomycosis must be considered in the development of diagnostic and therapeutic strategies.

• S044

**Worldwide Eye Banking (WEB) project: International survey of demand and supply**

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**Purpose** The whole eye banking (EB) process, from corneal retrieval to surgery, is improving. Paradoxically, corneal blindness worldwide is still increasing. The corneal supply is lagging far behind global demand. Few and partial works exist in this field. This has motivated our international survey: the Worldwide Eye Banking (WEB) project. Our final goal is to identify suitable solutions for countries willing to improve their corneal supply

**Methods** Design: A descriptive epidemiological worldwide transversal study. A questionnaire consisting of 23 items on keratoplasty and EB was designed. The data was collected from EB staff and corneal specialists via e-mailing, phone calls or direct interviews during international ophthalmology, eye research or EB congresses

**Results** Data was collected from more than 100 countries; covering a world population of 6.5 billions. Approximately 125 000 keratoplasties are performed each year from the 175 000 corneas collected. US EBs, first corneal exporter; use short-term storage while Europeans use long term organoculture. Lamellar keratoplasty is a new trend. Surgical indications have 2 profiles: infections for undeveloped countries; keratoconus and bullous keratopathy in industrial countries

**Conclusion** This demand/supply disparity is huge in many developing countries. Decreasing demand requires: 1) Trachoma fight in endemic zones 2) Iatrogenic edema prevention. Increasing supply requires: 1) Corneal donation politic dynamism 2) Local eye banking implantation in each country 3) Optimizing storage technique for better efficiency (retrieved/delivered graft ratio) 4) Bioengineering of endothelial graft. No financial interest for all authors

• S045

**Improvement of delivery of molecules into corneal endothelium using nanoparticles activated by femtosecond laser pulses**

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**Purpose** NanoFemtoTransfection (NFT) is an innovative and promising technique of gene transfer (Nature nanotechnology 2011). It consists in permeabilizing cell membrane with nanoparticles (NP) of black carbon activated by Ti-Sapphire femtosecond laser (FsL) in order to safely introduce molecule of interest. Initially described for cell suspension, we adapted during last year NFT to human corneal endothelial cells (EC) Aim: to further optimize NFT of EC *in vitro*

**Methods** The model of NFT of calcein (a small molecule) in an EC line (B4G12, DMSZ, Germany) was used to optimize exposition times, fluence and Ti-Saphir FsL beam movements in order to determine the best efficiency/toxicity ratio. Calcein transfer was observed with fluorescent microscopy (IX81, Olympus) and quantified by flow cytometry (FACSCalibur, BD, CA). Viability of EC was assessed by propidium iodide staining. Staining of actine with phalloidin and immunostaining of ZO-1 was performed to study cell alteration caused by NFT

**Results** With a static laser beam increasing FsL fluence and exposure time increased transfection efficiency but also EC mortality. The use of mobile FsL beam (with a XY micrometric stage, cheaper than a scanner), permitted an irradiation of the entire area of EC culture resulting in a noticeably increased efficiency that reached 38% of transfected EC and a limited toxicity (3%). Calcein positive EC presented the same phalloidin and ZO-1 patterns as control EC

**Conclusion** Optimized NFT parameters are currently being tested in our laboratory to transfect plasmids. Grants: Fondation pour la Recherche Médicale, Fondation des Aveugles de France

• S047

**Microbial keratitis after penetrating keratoplasty**

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**Purpose** To investigate the prevalence of microbial keratitis, predisposing risk factors, the spectrum of pathogens and the prognosis for graft survival and visual outcome in patients who developed microbial keratitis following penetrating keratoplasty (PK).

**Methods** We reviewed 14 cases (14 patients) of microbial keratitis after PK from January 1 2007, to December 31 2011. In all cases, corneal scrapings were obtained and microbiologically analyzed. Efficacy of treatment was evaluated by anatomical clarity of graft.

**Results** There were 100 PKs performed and 14 (1.4%) cases of culture-positive keratitis during the study period. Principal indications for PK were corneal perforation (6 cases) and pseudophakic bullous keratopathy (3 cases). Fifty per cent of infections occurred within 1 year of PK. Principal predisposing risk factors were suture-related problems (64.8%), epithelial defect (50%) and microbial keratitis in the previous graft (50%). Fifty of the scrapings were positive according to the microbiological evaluation with gram-positive cocci (42.85%), fungi (7.14%). We found 1 case of polymicrobial infection in renal graft. After medical and surgical treatments, 3 patients (21.42%) had a clear graft.

**Conclusion**: The development of bacterial keratitis after PK is a serious complication that is associated with a high incidence of graft failure and poor visual outcome. Postoperative control of risk factors and early recognition of infectious complications may decrease the incidence of severe microbial keratitis after PK.

• S046

**Biomechanical consequences of keratoconus treatments**

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**Purpose** to evaluate, biomechanical consequences of Crosslinking (CXL) in keratoconus, with intracorneal ring (ICR) or not, with a new instrument : Corvis © (Oculus).

**Methods** Our prospective study included ten patients with evolutive keratoconus (i.e. keratometric modification more than 0.5 D and/or refraction modification in 6 months), with pachymetry superior to 400 microns. For patients had both CXL and ICR (operated 3 months after CXL), six CXL only. The procedure was flash CXL (4 minutes of riboflavin, 15 minutes of UltraViolets). Patients were seen at 1, 3 and 6 months, with visual acuity, topography, corvis (deformation amplitude, applanation length, corneal velocity), videotopography.

**Results** The evaluation of corneal biomechanical properties of the cornea with corvis shows, after CXL, an increase of the applanation length of the second palanation pic : 0.8 pre-CXL, 1.02 post-CXL. Now, A2's length is usually decreased in keratoconus compared to normal cornea. Therefore, the A2 length change after CXL could be a parameter for the follow-up after CXL to show its efficacy.

**Conclusion** Corvis can be another tool in keratoconus' follow-up, based on the impacts of treatments (ICR and CXL) on biomechanical properties of the cornea.

• S048

**Viable endothelial cell density by triple HEC staining of a failed Descemet stripping automated endothelial keratoplasty**

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**Purpose** The rate of DSAEK failure decreases during learning curve. It has been previously reported that one of the main cause of rapid DSAEK failure was endothelial cell (EC) loss (Zhang, Arch Ophthalmol 2010). Aim: to report a DSAEK immediate failure and its innovative assessment with determination of the viability of residual EC

**Methods** In the eye bank, a cornea (donor: 44 years old, organ-culture: 23 days, ECD the day before cut: 2963 cells/mm<sup>2</sup>, deswelling 21h) was precut with a single pass of a 350µm head (Moria microkeratome). Lamella thickness was 199µm (ultrasound). The recipient had a Fuchs dystrophy. In the operating room, trephination with a Baron punch was eccentric but insertion was uneventful. The graft failed despite 2 rebubbling. A penetrating keratoplasty was performed 2 months later. During surgery the explanted lamella was immediately incubated with Hoechst 33342-Ethidium-Calcein-AM (HEC triple staining, Pipparelli, IOVS 2011) to determine the viability of residual EC. The thickness map of the lamella was then performed with an OCT (Casia, Tomey). The posterior surface of the recipient corneal button was observed with a microscope

**Results** The eccentric trephination caused an important step on 90° of circumference. Unplanned retention of Descemet membrane with guttae was found on the posterior face of the recipient cornea. The viable ECD of the explanted lamella was 585 cells/mm<sup>2</sup>. Keratocytes of both the lamella and the recipient cornea were viable

**Conclusion** Allogenic EC and keratocytes survive on a detached lamella at least during 2 months in the anterior chamber of the recipient. Grant: AOL 2012

### • S049

#### Corneal graft imaging by anterior segment OCT during storage in eye banks

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**Purpose** Endothelial graft pre-cutting by eye banks, rather than by surgeons in the operating room, is ongoing worldwide. A reliable and rapid thickness measurement before, during and after cut is an important parameter to guide the cut and assess the final result. Optical coherence tomography (OCT) can perfectly provide these data but commercial anterior segment-OCT are not fully adapted for the assessment of ex vivo corneas, especially organ-cultured ones that are not usually stored in viewing chambers contrary to 4°C stored corneas. Aim: we developed a simple device to adapt AS-OCT designed for patients to image corneas placed in petri dishes

**Methods** The device was developed for two AS-OCT (Heidelberg AS-slit lamp OCT and Tomey CASIA SS1000) and comprised only 3 pieces: 1/a mirror, held at 45 degrees, facing downwards, 2/the holder destined to receive the petri dish 3/the adaptor which was a plane that attaches the holder to the OCT head support. The petri dish containing the cornea was placed underneath the holder. The device was calibrated by comparing the measurements to those obtained holding the cornea vertical in front of the OCT in a 'physiologic' disposition

**Results** After calibration, thickness measurements using the device had a very good agreement ( $\pm 10 \mu\text{m}$ ) with measurements obtained without

**Conclusion** This device allows to easily adapt AS-OCT designed for medical use to the routine of eye banks using organ-cultured corneas that are not stored in specific containers. Grant: ANSM 2012, EFS 2011

### • S051

#### Long-term outcomes of penetrating keratoplasty in children

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**Purpose** Outcomes of pediatric penetrating keratoplasties (PK) performed for various corneal anomalies in children.

**Methods** Since 2000 56 PKs in children (6 months to 16 years) were performed. In infants group there were: 17 Peters Anomaly cases (24 eyes including 3 bilateral cases, 4 reoperations), 3 cases of sclerocornea. In older children surgeries were performed for 18 leucomas and 11 posttraumatic lesions. Follow up was at least 12 months. VA and graft transparency were analyzed.

**Results** 59.2% of infant's eyes had transparent corneas, VA ranged from 0.01 to 0.2. 19.2% of transplanted corneas were cloudy with blurred vision. Other grafts were failed including one phthisis. In older children success rate with transparent cornea was achieved in 82.7% of cases. Mean VA was  $0.3 \pm 0.12$ . Main complications were: immune rejection (11 eyes), stromal vascularisation (5 cases).

**Conclusion** Keratoplasty is an effective procedure to safe vision in children.

### • S050

#### Femtosecond laser cutting of multiple ultrathin corneal stromal lamellae for endothelial graft bioengineering

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**Purpose** The bioengineering of endothelial grafts (EG) is developing in several countries as an answer to the shortage of donor corneas worldwide. They are composed of a thin transparent biocompatible carrier evenly covered with tightly packed fully functional endothelial cells. Multiple options exist for the carriers. In our laboratory, we are focused on the optimization of human donor tissue because the well-organised cornea donation in France is not the most limiting point. Aim: to present the preparation of ultrathin stromal lamellae from a single human cornea

**Methods** Human organ-cultured corneas unsuitable for graft were used either with or without deswelling in Dextran containing medium. They were mounted on a artificial anterior chamber and cut with a femtosecond laser (femto LDV Z6, Ziemer) with aplanation. Six horizontal parallel cuts were realized on a 9 mm diameter area without vertical section. Cornea was then trephine at 8.00mm on a punch trephine. Thickness map was obtained before and after the cut with an AS-OCT equipped with a custom adapter for ex vivo corneas. Lamellae were separated under an operating video-microscope and again observed with OCT. Quality of surface were analysed with scanning electron microscopy and multiphotonic confocal microscopy with SHG for collagen fiber imaging

**Results** Seven regular lamellae of approximately  $100 \mu\text{m}$  were obtained on a single cornea and easily detached. Characterisation of surface quality is ongoing

**Conclusion** Femtosecond laser cutting of multiple lamellae on a single human cornea could allow obtaining a sufficient number of thin transparent and fully biocompatible carriers for EG bioengineering

### • S052

#### Intrastromal corneal rings and corneal collagen crosslinking for progressive keratoconus: Comparison of 2 sequences

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**Purpose** To compare 2 sequences of combined intrastromal corneal ring segment (ICRS) implantation and corneal collagen crosslinking (CXL) in progressive keratoconus.

**Methods** In this retrospective study, CXL was followed by ICRS implantation (group A) or ICRS implantation was followed by CXL (group B). Uncorrected (UDVA) and corrected (CDVA) distance visual acuities, spherical equivalent (SE), manifest cylinder and mean keratometry (K) were compared preoperatively and postoperatively.

**Results** 17 eyes of 10 patients with progressive keratoconus were included in this study, 11 eyes in group A and 6 in group B. The mean interval between treatments was  $4.3 \pm 2.9$  months in group A and  $5 \pm 4.4$  months in group B. The mean age is 27.6 years in group A and 28.4 years in group B. The 2 groups are equivalent preoperatively. The mean UDVA and CDVA improved in both groups (UDVA:  $0.50 \pm 0.22$  to  $0.60 \pm 0.20$ , group A, and  $0.28 \pm 0.19$  to  $0.62 \pm 0.22$  (p<0.05), group B; CDVA:  $0.60 \pm 0.15$  to  $0.70 \pm 0.14$  and  $0.70 \pm 0.14$  to  $0.88 \pm 0.17$  respectively). The cylinder and mean K values decreased in both groups (cylinder:  $-3.63 \pm 1.27$  to  $-0.95 \pm 1.54$  (p<0.05), group A and  $-3.60 \pm 1.85$  to  $-2.80 \pm 2.19$  group B; mean K:  $44.8 \pm 1.45$  to  $42.8 \pm 1.7$  (p<0.05) and  $43.98 \pm 1.74$  to  $43.8 \pm 1.8$ , respectively). SE: values decreased in group A ( $-3.4 \pm 1.5$  to  $-1.3 \pm 1.4$  (p<0.05)) instead increased in group B ( $-1.1 \pm 0.8$  to  $-1.7 \pm 1.0$ ). Overall, the 2 sequences observed show an improvement in UDVA, cylinder and mean K.

**Conclusion** In our study, there is no statistical difference between the 2 sequences: ICRS implantation followed by CXL or CXL followed by ICRS implantation.

• S053

**Ferrara ring's effects on corneal asphericity in keratoconus treatment**

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**Purpose** In keratoconus treatment, Ferrara' rings nomogram integrates the Q factor defining corneal asphericity. Our study deals with the effect of the implantation of Ferrara rings on the Q factor

**Methods** This retrospective and monocentric study involves forty eyes of thirty-one patients suffering from keratoconus implanted with Ferrara' rings, with an uncorrected visual activity (UCVA), a best spectacle-corrected visual acuity (BSCVA) and a videokeratography, before and six months after surgery. The items followed are the UCVA, the BSCA, the average (Kmean) and maximum keratometry (Kmax) and the Q factor in the central 20° (Q20) and 40° (Q40). The analyse of the visual acuity (more or less than a line) enables to differentiate two groups of "good" and "bad" responders. The statistical tests are a Wilcoxon signed rank for pre and post operative comparisons, a Wilcoxon-Mann Whitney for group comparisons, and a Spearman for correlations.

**Results** The average age of patients is 32,8 years with 83,1% of male gender. Twelve eyes were implanted with two rings and twenty-eight with one. We find an average gain of 3,6 lines for UCVA, 1,1 lines for BSCVA and a decrease in Kmean of 1,4 dioptre. Q20 and Q40 are meaningly improved after surgery, but there is no significant difference between the studied groups. Lastly, before surgery, BSCVA is correlated to Q20, and Kmax to Q40. After surgery, UCVA is correlated to Q20 and Kmax to Q20. But the Kmax variation and the UCVA or BSCVA gains are not correlated.

**Conclusion** Including the Q factor in the nomogram establishing ring's choice improves the keratometric field but not the visual outcome.

• S054

**Intracorneal foreign body in the confocal microscopy in vivo images**

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**Purpose** Presentation of a patient with intreacorneal foreign body, differentiation in images of corneal confocal microscopy in vivo.

**Methods** The patient admitted because of suspicion of post-traumatic epithelial ingrown syndrome, to diagnosis and treatment, the following tests were performed: basic slit lamp examination, corneal scans using Scheimpflug camera (Pentacam, Oculus) and corneal confocal microscopy in vivo (Rostock Cornea Module, Heidelberg Engineering Retina tomograph III).

**Results** On a slit lamp examination we described macroscopic limited opacity without evidence of inflammation. In the images from the Scheimpflug camera - hiperreflective deposits obscuring other structures of the cornea. The corneal confocal microscopy images describes post-traumatic corneal scar changes with clearly marked wound after the foreign body intake and single round conjunctival epithelial cells within the corneal stroma. In addition activation of keratocytes was observed. On about 350 microns depth, small deposits of number from few to several, with increased linear streak hyperreflex and large, organized foreign body (piece of glass) of 1.5x0.7 mm diameter, irregular margins, with a similar hiperreflex.

**Conclusion** Corneal confocal microscopy is a useful tool for the detection and differentiation of corneal opacities of uncertain etiology.

• S055

**Kyrieleis plaques in herpes zoster virus associated acute retinal necrosis**

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**Purpose** To present a case of unilateral acute retinal necrosis syndrome (ARNS) with associated Kyrieleis plaques.

**Methods** A 56 year old male with recently diagnosed ARNS with necrotic lesions, extensive retinitis 360° around the arcades and multifocal lesions presented with decreased vision and floaters in his left eye. Anterior uveitis of 3+ cells, with mutton fat keratic precipitates and vitritis 2+ with severe haze settled down following anti-viral and steroid treatment. Polymerase chain reaction from anterior chamber tap revealed presence of Herpes Zoster Virus.

**Results** Kyrieleis plaques and segmented perierteritis appeared at presentation and spreaded in the first 2-3 weeks whilst inflammation was settling down. They affected the majority of retinal arteries near the optic disc; however there was no evidence of sheathing of the arteries wall on fluorescein angiography.

**Conclusion** Increased doses of steroids appear to control the inflammation. There was no association found between Kyrieleis plaques and severity of inflammation.

• S056

**Cytomegalovirus retinitis (CMVR)-related retinal detachment (RD) surgery in AIDS patients in an Asian population**

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**Purpose** This study reports the outcomes of retinal re-attachment surgery in patients with Cytomegalovirus retinitis (CMVR)-related retinal detachment (RD) in an Asian population.

**Methods** Review of CMVR characteristics, surgical outcomes and complications in 19 patients with CMVR-related RD who underwent surgery from January 2000 to June 2011.

**Results** CMVR was inactive in 73.7% of the patients at the time of surgery. Fourteen (73.7%) of RDs were macula-off and 4 (21.1%) patients had proliferative vitreoretinopathy (PVR). Anatomical success was achieved in 14 patients (73.6%). Eight patients (42.1%) had improvement of 1 or more lines in BCVA, 7 patients (36.8%) maintained BCVA and 4 patients (21.1%) had poorer BCVA at 6 months after surgery. Of the 13 patients who presented with worse than 6/120 vision, 30.8% regained ambulatory vision or better. Five patients had re-detachments (26.3%). Six phakic patients (54.5%) developed cataracts post-surgery at a median interval of 6 months. Seven patients had immune recovery uveitis (IRU) prior to RD. Median durations from CMVR and IRU diagnoses to RD were 2.0 and 1.0 months respectively.

**Conclusion** Surgery for CMVR-related RD is associated with good anatomical outcomes with most patients maintaining or improving vision. Factors related to better outcomes include smaller CMVR lesion size and the absence of PVR. CD4 counts were not related outcomes. Patients diagnosed with CMVR and IRU require close monitoring for development of RD.

• S057

**High doses of fatty acids for reducing cystoid macular oedema secondary to inflammation or branch retinal vein occlusion**

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**Purpose** To evaluate the effect of high doses EPA/DHA for patients with cystoid macular oedema.

**Methods** 14 eyes with cystoid macular oedema (8 secondary to chronic inflammation and 6 secondary to branch retinal vein occlusion) were treated with 5g/day of EPA/DHA. Visual acuity and macular oedema results from OCT scans were noted.

**Results** The mean macular oedema was reduced from 564 $\mu$ m to 243 $\mu$ m in 12 months. The mean visual acuity gain at 12 months was 2.85 lines. No anti-VEGF injections were given during treatment with high doses EPA/DHA.

**Conclusion** Innovative therapy of using high doses of fatty acids to reduce macular oedema and improve vision in patients with cystoid macular oedema secondary to inflammation or branch retinal vein occlusion.

• S058

**The regulatory effects of 1,  $\alpha$ , 25-dihydroxyvitamin D3 on the expression of inflammatory cytokines in diabetic retinopathy**

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**Purpose** Diabetic retinopathy (DR) is typically characterized by abnormal changes in the microstructure of retina as a result of prolonged uncontrolled diabetes mellitus. Several mechanisms on how high glucose concentration alters retina microvasculature have been proposed. One of these mechanisms is the activation of alternative glucose pathways leading to accumulation of several metabolic end-products which induce oxidative stress in retina causing cellular changes of the expression of pro and inflammatory mediators. Thus, the aim of this study is to investigate the protective function of vitamin D in diabetic retinopathy

**Methods** 1-Treatment of ARPE-19 cell line with the active form of vitamin D2-Diabetic animal models are induced for type 1 diabetes mellitus and treated with calcitriol

**Results** 1-Vitamin D down-regulates the expression of pro and inflammatory cytokines involved in DR 2-It up-regulates the expression of antioxidative stress scavengers 3-Vitamin D improves cell survival during inflammatory process

**Conclusion** Changes in the expression patterns of immunomodulatory and antioxidative proteins after vitamin D treatment propose a protective function of vitamin D in DR

• S059

**Detection of antibodies against homologous Mycobacterium avium subspecies paratuberculosis and beta-cell antigen zinc transporter 8 epitopes in Sardinian type 1 diabetic patients with proliferative diabetic retinopathy**

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**Purpose** Diabetic retinopathy (DR) is the leading cause of new cases of blindness in developed countries. ZnT8, a beta-cell membrane protein involved in Zn<sup>++</sup> transportation, may act as a major autoantigen in Type 1 Diabetes (T1D). Dysregulation in Zn<sup>++</sup> homeostasis has been implicated in the pathogenesis of ischemia in DR. Mycobacterium avium subspecies paratuberculosis (MAP) causes an asymptomatic human infection transmitted from dairy herds through food contamination. MAP3865c, a MAP cell membrane protein, has been shown to display a relevant sequence homology with ZnT8. Moreover, antibodies recognizing MAP3865c epitopes have been found to cross-react with ZnT8 in T1D patients. The purpose of this study was to detect serum antibodies against 6 highly immunogenic MAP3865c epitopes in T1D patients with proliferative diabetic retinopathy (PDR) and in healthy controls (HCs).

**Methods** Blood samples were obtained from 23 T1D patients with PDR and 39 HCs. Antibodies against 2 trans-membrane (MAP3865c125–133 MAP3865c133–141) and 4 C terminal (MAP3865c246–252, MAP3865c256–262, MAP3865c261–267 and MAP3865c281–287) peptides were detected by indirect ELISA. Fisher's exact test and ROC curves were used to assess results.

**Results** Antibodies against MAP3865c peptides were found in 6 out of 23 (26%) T1D patients with PDR and 2 out of 39 (5%) HCs, a statistically significant difference ( $p=0.04$ ).

**Conclusion** Results suggest that serum antibodies against MAP3865c peptides may play a role in the pathogenesis of PDR in T1D patients. Further larger studies are necessary to confirm these preliminary data.

• S060

**Uveitis and lyme disease: About two case reports**

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**Purpose** Lyme disease (LD) is a spirochete anthroponosis transmitted by tick bite. The ocular manifestations are diverse although rare. Uveitis is one of the most frequent.

**Methods** Two patients 46 and 52 years, referred for unlabeled and steroid dependent uveitis were studied. The first had two previous episodes of uveitis straight herpes diagnosed in early 2011. The second was treated with oral corticosteroids for chronic bilateral panuveitis evolving since February 2010, with a first anterior uveitis in 1989 (unavailable negative test). Laboratory tests with viral serology Quantiferon, IDR, syphilis serology and FAN have been made.

**Results** The first patient had a visual acuity preserved, bilateral vitritis and a right anterior uveitis with pigmented keratic precipitates. The fundus and OCT were normal, angiography showed a left papillary hyperfluorescence. The autoimmune hypothesis had been raised with centromere antibody positive before having a positive IgM ELISA Lyme serology confirmed by a Western Blot (WB). The second patient had bilateral visual loss, right ocular hypertension associated with diffuse bilateral anterior synechiae on a plot of uncontrolled glaucoma. There was a serous retinal detachment in right fundus. The B-scan ultrasonography showed bilateral posterior scleritis. She was treated with solumedrol before the positive IgG ELISA and WB. No tick bite was mentioned, but walks in the forest were found.

**Conclusion** The diagnosis of Lyme disease must be suspected in the face of recurrent uveitis even without a tick bite. Specific serology may be positive if completed, allowing antimicrobial therapy.

• S061

**Can we consider syphilitic uveitis as neurosyphilis? A retrospective analysis of lumbar puncture results in a cohort of syphilitic uveitis patients**

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**Purpose** To investigate the cerebral spinal fluid (CSF) composition of patients with syphilitic uveitis (SU) and classify SU as neurosyphilis or not.

**Methods** Retrospective study of SU patients from 2 Belgian university centers from 2003 to 2012. The diagnosis of SU was based on a positive syphilitic serology (FTA-Abs and RPR) and a treatment response. Charts were reviewed for uveitis anatomical location, HIV status, serum RPR and CSF findings. Diagnosis criteria for neurosyphilis were: positive CSF-RPR and/or CSF-white blood cells (WBC)  $\geq 5/\mu\text{l}$  for HIV-, and positive CSF-RPR and/or CSF-WBC  $\geq 20/\mu\text{l}$  for HIV+.

**Results** 20 patients were included. 5 were HIV+. There were 3 papillitis, 10 posterior uveitis and 7 panuveitis. Cells were found in CSF in all patients (mean  $40/\mu\text{l}$ ) and were predominantly lymphocytes. Mean CSF-proteins, lactate and glucose were  $0.57\text{g/l}$ ,  $17\text{mg/dl}$  and  $62\text{mg/dl}$  respectively. No differences were found between HIV+ and - patients. CSF-RPR was + in 2/5 (40%) of HIV+ and in 11/15 (73%) of HIV- with no statistical difference.

**Conclusion** In our series, all patients had a CSF cellular reaction, mostly lymphocytic. This strongly suggests that the blood brain barrier is disrupted during syphilitic uveitis. However, a positive RPR was not observed in all CSF. Despite this discrepancy, following our criteria, 100% of our patients could be classified as neurosyphilis.

• S062

**Persistent macular edema with secondary neovascular membrane following a panuveitis in woman with Behcet's disease resolved by a switching to aflibercept injections**

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**Purpose** To report the effective treatment of a neovascular membrane following recurrent panuveitis associated to Behcet's Disease, in a woman no responders to cyclosporine and biological response modifiers, refractory to ranibizumab, who was switched to aflibercept injections.

**Methods** An interventional case report with optical coherence tomography (OCT) scans.

**Results** A 56-year-old Caucasian woman was suffering from recurrent panuveitis on both eyes for Behcet's Disease with a neovascular membrane on left eye. She had undergone one entire cycle of therapy with cyclosporine ( $5\text{mg/Kg/die}$ ) and two infusion of infliximab which resolved panuveitis, without functional or anatomical improvement of the eye clinic on the left eye. Six months after the second infusion of infliximab, she received two consecutive intravitreal injections of ranibizumab with the persistent of the neovascular activity and without any improvement of visual acuity (VA). Four weeks following the second injection, the treatment was switched to two aflibercept injections. Two weeks later, her best-corrected visual acuity improved from 0.4 to 0.7. Macular edema resolved with a reduction of central retinal thickness from  $398\text{ }\mu\text{m}$  before Aflibercept injections to  $274\text{ }\mu\text{m}$  after Aflibercept injections, as measured by OCT scan.

**Conclusion** Aflibercept could be a new therapeutic, effective, safe, local treatment option in patients with complicated noninfectious uveitis as Behcet's Disease, no responders to immunosuppressive agents or immunobiological response modifiers and previous anti-VEGF treatments.

• S063

**Clinical characteristics of acute retinal pigment epitheliitis: Case series**

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**Purpose** To describe characteristics of acute retinal pigment epitheliitis by analyzing 16 cases.

**Methods** Retrospective chart review of patient diagnosed with acute retinal pigment epitheliitis was done. Medical records, fundus photo, optical coherence tomographic(OCT) findings and fluorescein angiographic(FAG) findings were reviewed.

**Results** The average age at diagnosis was 39.6 year-old, with male to female ratio of 2:1. Acute metamorphopsia or central scotoma with slight decreased vision were chief complaints, and the symptoms resolved spontaneously after 60.1 days. Funduscopic finding revealed macular mottling with surrounding yellow hypopigmented areas, with subsensory retinal detachment in some cases. Disruption of the photoreceptors' inner segment and outer segment interface with hyperreflection at retinal pigment epithelial(RPE) level were found in OCT. On FAG, window defect was seen. Retinal pigment epithelial changes remained after resolution of symptoms in all cases.

**Conclusion** Acute retinal pigment epitheliitis is an infrequent macular disorder in young adults with symptoms of metamorphopsia or central scotoma and signs of RPE change with window defect on FAG. Differential diagnosis is important because of the good prognosis without treatment.

• S064

**Adaptive optics imaging of retinal vasculitis**

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**Purpose** To document perivascular infiltrates in the retina using high resolution flood illumination adaptive optics (AO) imaging in patients with retinal vasculitis.

**Methods** The charts of five subjects with retinal vasculitis showing evidence of perivascular infiltrates by means of AO imaging (rtx1 camera, Imagine Eye, Orsay, France) were reviewed.

**Results** Diagnoses included primary idiopathic retinal vasculitis (n=1), Lyme's disease (n=2), TB related retinal vasculitis (n=1) and idiopathic retinitis, vasculitis and aneurysm (IRVN; n=1). Using AO imaging, perivascular infiltrates appeared as discrete, linear areas of opacification along the veins, often surrounding the venous narrowing. AO imaging was more sensitive than either fundus photographs or fluorescein angiography for their detection. Follow-up examinations (n=3) showed a changing pattern of narrowing of the vessel lumen, and eventual disappearance of these perivascular infiltrates. In the IRVN case, inflammatory infiltrates were found around arterioles and veins as well, suggesting an inflammation induced-phenomenon.

**Conclusion** AO imaging has a higher sensitivity than fundus photographs or fluorescein angiography to detect perivascular inflammatory infiltrates. Vascular inflammation is strongly linked to changes in vascular lumen diameter. AO imaging may have a particular interest when diagnosis and following subjects with retinal vasculitis.

• S065

**Hyperreflective dots detected in the retina using spectral domain optical coherence tomography**

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**Purpose** to investigate morphologic changes in posterior segment diseases using spectral-domain optical coherence tomography (SD-OCT). To discuss the role and characteristics of hyperreflective dots (HRD) in retinal inflammatory diseases and choroidal neovascularization (CNV).

**Methods** 3 patients (3 eyes) with posterior segment diseases (Lyme disease, Cat scratch disease, and suspected Best vitelliform macular dystrophy with CNV) were retrospectively reviewed. Thorough ophthalmologic examination and SD-OCT were performed during the first visit and follow-up. HRD behavior was evaluated in the retina with SD-OCT pre- and post-treatment.

**Results** in all three eyes, various amounts of HRD were detected in the foveal or parafoveal area and, in one case, near the optic disc prior to the treatment. Hyperreflective dots accumulated densely, especially surrounding the area of fluid accumulation. During follow-up, progressive resolution of the hyperreflective dots was observed with SD-OCT. HRD reduction was observed mainly in cases of complete macular edema resolution and correlated greatly with visual acuity (VA) improvement.

**Conclusion** HRD seen with SD-OCT in retinal inflammatory diseases behave similarly as in wet age-related macular degeneration and diabetic maculopathy. The association between VA improvement and a decrease in the amounts of HRD could be used as a helpful treatment indicator in both inflammatory posterior segment diseases and choroidal neovascularization.

• S066

**Swept source-optical coherence tomography analysis of choroidal involvement in patients with uveitis**

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**Purpose** Swept source OCT (SS-OCT) is currently the best visualization technique of the choroid. The aim of our study is to describe its thickness and vascular structures in patients with choroiditis.

**Methods** We have completed a choroidal map with SS-OCT, using 12-mm horizontal vertical scans through the macula, for a group of patients with choroiditis, referred to Pitie Hospital, from October 2012 to March 2013.

**Results** Eight patients have been included with a sex ratio of 1, an average age of 56.5 years : 5 birdshot retinochoroidopathies (BRC), 1 multifocal choroiditis , 1 Vogt-Koyanagi-Harada (VKH) disease, 1 acute posterior placoid chorioretinitis concomitant with a syphilitic seroconversion. In four active BRC, the average choroidal thickness was increased to 339 µm (389 µm ±41.93), among whom one BRC patient experienced a decrease of 30 µm in choroidal thickness immediately after corticotherapy. On the contrary the fifth patient, an advanced form of BRC, showed signs of atrophy with a choroidal thickness of 194 µm. Choroidal thickness in the VKH patient was unchanged in both eyes. There was a significant asymmetry in the patient with syphilitic chorioretinitis, with a choroidal thickness of 334 µm in the affected eye versus 387 µm in the contralateral eye. Yet, we observed that Haller's layer of the affected eye was harder to visualize, due to possible inflammatory infiltrations

**Conclusion** These choroidal maps are among the first choroiditis cases explored by SS-OCT, first step of a mapping project which we wish to amplify in order to determine a possible threshold of choroidal thickness indicating a subclinical relapse. This could make SS-OCT an additional tool besides ICG angiography for the exploration of the choroid.

• S067 / 4455

**Mydriaser pupillary dilation for cataract surgery:**

**A clinical and economic study**

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**Purpose** Mydriaser<sup>\*</sup> is an ophthalmic insert containing phenylephrine and tropicamide. A prospective clinical evaluation of Mydriaser for pupillary dilatation prior to cataract surgery was conducted. The impact on nurse time of using Mydriaser compared to eye drops was also evaluated.

**Methods** The following data were collected prospectively: amount of time spent inserting Mydriaser, patient satisfaction score (on a scale of 1-10), pupil size (on a pre-defined categorical scale of 1 (small pupil) to 4 (very large pupil)) and any adverse events related to the insert. An economic model of mydriasis was also developed.

**Results** Mean nursing time spent inserting Mydriaser was 1.52 minutes (SD=0.6, n=85), mean patient satisfaction score was 8.97 (SD=2.32, n=97) and mean pupil size was 3.32 (SD=0.66, n=114) corresponding to a large to very large pupil size. The insert had extruded unnoticed in 3 cases. For a national population (England), the estimated nurse time saved was 1,159 hours per week when using the single application Mydriaser compared to repeated instillation of eye drops to achieve mydriasis.

**Conclusion** Mydriaser is a safe and efficacious alternative to topical drops for pupillary dilatation. The rate of insert extrusion was low compared to that published in a smaller study reporting on a much smaller sample. This may reflect a learning curve for Mydriaser insertion. The economic analysis demonstrated that using Mydriaser could potentially free up nursing time to spend on other responsibilities.

**Commercial interest**

• S068 / 4456

**Cataract surgery and retinal vein occlusion:**

**Is there an association?**

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**Purpose** To describe and characterise patients who were noted to have developed postoperative retinal vein occlusion (RVO) soon after cataract surgery and to hypothesise the underlying mechanism of such manifestation

**Methods** All the patients who developed post-operative retinal vein occlusion (RVO) soon after cataract surgery between April 2012 and April 2013 in Cumberland Infirmary, Carlisle, United Kingdom, were consecutively recruited. All cataract surgeries were performed under topical anaesthesia. All the patients were assessed pre-operatively and 4 weeks after the cataract surgery.

**Results** A total of 3 patients were included in this study. All 3 patients were male and mean age of the cohort was 82 ± 10 years. The mean preoperative best-corrected visual acuity (BCVA) of the cohort was 78.3 ETDRS letters. Vascular risk factors were identified in all patients, including previous RVO (66%), hypertension (100%), diabetes (33%) ischemic heart disease (33%) and smoking (66%). All patients underwent phacoemulsification with IOL implantation without any complication. Time interval between surgery and diagnosis of RVO was 73.3 ± 39.7 days. Postoperative BCVA of the cohort was 50 ETDRS letters. Two patients required 0.7mg intravitreal dexamethasone implant (Ozurdex).

**Conclusion** Vascular risk factors were identified in all the patients who were noted to develop RVO following cataract surgery. We hypothesise that the fluctuation of intraocular pressure during cataract surgery predisposes to the development of intraoperative retinal vein occlusion in our cohort. Preoperative counselling regarding the risk of RVO before the cataract surgery may be indicated.

## • S069

**Risk factors for intraoperative floppy iris syndrome: One year prospective study**

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**Purpose** Intraoperative Floppy Iris Syndrome (IFIS) was associated with a-blockers use. Our purpose was to evaluate risk factors for IFIS in patients undergoing phacoemulsification.

**Methods** Participants in our study were 1024 patients, who underwent routine phacoemulsification cataract surgery. The following data were recorded and evaluated as possible risk factors for IFIS: ophthalmological conditions (glaucoma, pseudoexfoliation, age-related macular degeneration), sociodemographic features, axial length, hypertension, diabetes mellitus and medications being taken at the time of surgery. Cases were characterized intraoperatively as IFIS and non-IFIS. Univariate and multivariate logistic regression was performed with SPSS 19.0.

**Results** IFIS was observed in 61/1024 eyes (5.9%, 95%CI: 4.3%-7.2%). Current use of tamsulosin, alfuzosin, terazosin, benzodiazepines, quetiapine and finasteride were all independently associated with IFIS. Borderline associations were noted for rivastigmine. Hypertension and short axial length were also significantly associated with IFIS. It is worthy to note that IFIS was not associated with the duration of a-blockers intake.

**Conclusion** Apart from the well-established associations with alpha1-adrenergic receptor antagonists, this prospective study points to benzodiazepines, quetiapine, hypertension and short axial length as potential risk factors for IFIS.

## • S070

**Cataract and uveitis: Comparison of two different anti inflammatory protocols for the prevention of post-operative complications**

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**Purpose** In the context of cataract surgery in patients followed for chronic uveitis, evaluate the effectiveness of a standart protocol corticosteroids versus a simplified protocol, by comparing the visual acuity and inflammation pre-and postoperatively in each two groups.

**Methods** Prospective non-randomized single-center study was conducted in 2012, including 35 eyes(30 patients), mean age was 54.9 years. Patients were followed for chronic uveitis, whatever the cause, and were eligibles for a cataract surgery. 20 eyes received the standart protocol of corticosteroids, which was oral corticosteroids combined pre-and postoperatively, as well as bolus injection of solumedrol and subconjunctival injection of dexamethasone in peroperative time. On the other side, 15 eyes received a lightweight protocol comprising only intraoperative corticosteroids (bolus and subconjunctival injection) without oral corticosteroids.

**Results** With a median follow up of 6 months, after surgery, gain of visual acuity was significant in all patient. We lamented 4 earlier acute inflammatory relapses , 2 postoperative macular edema, 1 epimacular membrane and 1 retinal detachment. These complications did not link significativly with the choice of protocol, completeness or light. They were put in relation with the severity of previous inflammation, and particularly his etiology , preoperative inflammatory state, and if it was an antecedent of macular edema or not.

**Conclusion** Simplified protocol can be proposed for cataract surgery in patient with chronic uveitis, especially for patients who did not relapse over a year and with no history of macular edema, as well as patients who were not found obvious systemic cause

## • S071

**Cataractogenesis and surgical outcome in patients with uveitis**

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**Purpose** To compare the risk of cataract development, the surgical management and the prevalence of complications in 2 groups of patients with uveitis treated in 2000 and 2010.

**Methods** Retrospective study of patients with uveitis referred to an academic centre for the diagnosis and treatment of uveitis. An extensive work-up based on clinical presentation was performed when necessary. Corticosteroids were the mainstay of therapy in 2000 but new immunosuppressive molecules and biologics were used in 2010. Two groups were analyzed based on the year of cataract surgery (group A:2000 and group B:2010). Demographic factors, delay between onset of uveitis and cataract surgery, improvement of visual acuity and major complications were studied. Phacoemulsification and intraocular lens implantation were performed in all cases respecting a 3-month quiescence of uveitis and an efficient perioperative anti-inflammatory regimen.

**Results** The study included 125 eyes of 107 patients. 59 eyes (48 patients: F/M: 1.29) were operated in 2000 (group A) and 66 (59 patients: F/M: 1.5) in 2010 (group B). An associated condition was determined in 81 cases (75.5 %): mainly herpetic uveitis (9), sarcoidosis (7), tuberculosis (10), birdshot chorioretinopathy (7), Behcet's disease (8), spondyloarthropathy (10), Vogt-Koyanagi-Harada disease (5), Fuchs heterochromic iridocyclitis (8). Delay between onset of uveitis and surgery was 58.86 months in group A and 111.68 months in group B ( $p<0.01$ ). Mean BCVA postoperatively improved from logMAR 0.927 and 0.731 to 0.439 and 0.242 in groups A and B, respectively.

**Conclusion** During a 10-year period, management of uveitis has dramatically improved. Better management of ocular inflammation based on biologic agents may delay cataractogenesis in active patients.

## • S072

**Femtosecond laser capsulotomy versus manual technique: A clinico-morphological study of the resected capsules**

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**Purpose** To compare resected lens capsules created by femtosecond laser system with the conventional manual techniques.

**Methods** A prospective, observational case series of 6 patients who underwent capsulotomies performed by an optical coherence tomography-guided femtosecond laser compared with 6 patients which capsulotomies created with the conventional manual technique. The resected capsules were evaluated histologically. Electron microscopy was performed to examine their capsule edge.

**Results** Histologically the capsule edges produced by manual were smooth and continuous however performed by femtolaser the capsule edges showed small microgrooves in high magnification. Electronmicroscopically these microgrooves presented as pointed serrated structure like a lacerated jeans.

**Conclusion** The femtosecond laser produced capsulotomies were clinically more precise in diameter and in size however their capsules showed irregular stripped edges than those created with the conventional manual technique. The microgrooves were produced by the laser pulses. A certain weakness of the laser produced edges is to expect and needs more clinical experiences.

• S073 / 4457

**Morphological and proliferative studies on ex vivo cultured human anterior capsule lens epithelial cells**

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**Purpose** The proliferation and migration of lens epithelial cells (LECs) that remain in the capsular bag after cataract surgery can lead to development of posterior capsule opacification (PCO) - a major cause of post-operative visual loss. The multipotency of anterior lens capsule (LC) LECs is still controversial. We aimed to test the proliferating and migrating potential and to characterize structurally the ex vivo cultured LECs growing out of human anterior LC.

**Methods** The explants of LCs were obtained from uneventful cataract surgery and consisted of basal membrane and monolayer of LECs that were cultivated under adherent conditions. The size and shape of the outgrowing cells were recorded by scanning electron microscopy (SEM) and their migration and proliferation potential were followed on a daily basis throughout their continued growth using light microscope.

**Results** The migration and proliferation of anterior LC LECs filling up the denuded region of the LC could be followed by light microscopy. These cells could also migrate to the opposite side of the capsule as shown by both, light microscopy and SEM. The migration and proliferation of LECs on glass or plastic culture surfaces could be shown by both techniques, while the distribution of the LECs and their morphology could be analyzed in details by SEM.

**Conclusion** Classic light microscopy and SEM studies can be used to show that human anterior LC harbors LECs that can migrate and proliferate, suggesting their multipotency and putative stem cell nature. The anterior LC explants can be used to study PCO and the potential of different pharmaceutical or physical treatments against PCO development.

• S075

**Active caspase-3 expression in healthy lens epithelium**

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**Purpose** To determine the distribution and localization of active caspase-3 in normal rat lens epithelium by immunohistochemistry.

**Methods** Altogether, 40 Sprague Dawley rats were sacrificed and eye lenses were removed. One eye from each animal was fixed and then stored at -80°C. Three mid-sagittal sections of each lens were processed for immunohistochemistry. Lens epithelial cell position was identified and specified as its consecutive position in relation to the first nucleus counted. The counting started from one nuclear bow and ended in the opposite side nuclear bow. The nuclei labelled with active caspase-3 and their relative position was identified three times, counts, in each section. Then, each lens was divided into fifteen segments and active caspase-3 labelling was calculated in each segment.

**Results** Active caspase-3 was more expressed in the anterior pole of the lens with the of 37 % active caspase-3 labelled cells. The variance for animals, sections and countings was estimated to 11, 3 and 83 %, respectively. Fitting the fraction of labelling to a double exponential model, as a function of segment number starting from the anterior pole, assuming an exponential decrease from the anterior pole toward periphery, resulted in a 95 % confidence interval for decrease rate ( $1/k$ ) =  $-1.5 \pm 0.2$  segments and inflection point =  $7.7 \pm 0.2$  segments

**Conclusion** Active caspase-3 is present in the normal lens, with a maximum activity around the anterior pole that tapers off towards the periphery. Quantitative analysis of immunofluorescence of active caspase-3 requires at least 3 sections per lens. The number of countings of labelled nuclei is the limiting factor for the precision in an estimate of fraction of labelling.

• S074

**Transporters of glycine, cystine, glutamate and glutamine in canine lens epithelial cells**

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**Purpose** The aim of this study is to evaluate GSH synthesis-related amino acid (glycine, cystine, glutamate and glutamine) transport activity in a canine lens epithelial cell line (cdLEC) and define the transporter.

**Methods** The primary lens epithelial cells of mature cataract dog were transfected with the expression plasmid DNA of large T antigen from replication origin-defective simian virus 40 (SV40), then cloned using glass cylinder. The transport activities of four amino acids in lens epithelial cells were determined. The cDNA sequences of transporter contributing GSH synthesis were also determined.

**Results** Na-dependent glycine and glutamate transport activity was  $2.70 \pm 0.26$  and  $4.40 \pm 1.10$  nmol/mg protein min, respectively. The cystine transport was Na-independent and the transport activity was  $0.626 \pm 0.17$  nmol/mg protein min. The cDNA sequence of the glycine transporter 1(GlyT1), cystine/glutamate exchanger (xCT<sub>i</sub>), Na-dependent neutral amino acid transporter 2(ASCT2), and glutamate transporter (EAAT) were determined. Western blot analysis confirmed their expression in membrane protein sample of cdLEC.

**Conclusion** Lens epithelial cells possess GlyT1, xCT, ASCT2 and EAAT1-5.

• S076

**In vivo analysis of protein quality control in response to aging using transgenic mice**

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**Purpose** It has been suggested that aging affects the cells ability to protect protein integrity, which is essential for cellular homeostasis. On cell aging there is impaired protein homeostasis, which can lead to protein insolubilization that is toxic to cells. The carboxyl terminus of Hsp70-interacting protein (CHIP) is an important ubiquitin ligase for protein quality control, and particularly in selective degradation of aggregation prone proteins. The objective of this study was to characterize the transgenic mice that overexpress CHIP/Ubc5 in lens epithelial cells and investigate the role of these proteins in the process that maintains proteins in solution and therefore participates in a number of age-related diseases, including cataract.

**Methods** To overexpress CHIP, the  $\alpha$ -crystallin promoter was used to drive the expression of CHIP-Ubc5 in lens epithelial cells of transgenic mice. The CHIP levels of transgenic mice (Tg) were determined with western blotting analysis. Cataract was induced by single subcutaneous injection of sodium selenite and the lens transparency was assessed by slit lamp examination. The soluble and insoluble protein content in all groups was evaluated.

**Results** The data show that the transgenic mice only overexpress the transgene in lens epithelial cells. The slit lamp analysis showed that transgenic mice appears developed less cataract compared with wild type, with concomitantly increase in ratio of the soluble and insoluble proteins.

**Conclusion** Together the data suggest that overexpression of CHIP/Ubc5 in lens epithelial cells could prevent cataract formation by promoting reduction of protein insolubilization.

• S077

**The effects of actomyosin inhibitors on cytoskeletal distributions in the lens**

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**Purpose** Previous work indicates that inhibitors of actin, myosin, and myosin light chain kinase are associated with a softening of the lens. The softening of lenses did not affect the optical quality, despite the application of inhibitors that, based on Western blot analysis, affect the cytoskeleton of lens fibre cells. Inhibitors can impart their effects biochemically or structurally; therefore, in the present study, the cytoskeletal distribution of inhibitor-treated lenses was examined using confocal microscopy.

**Methods** Lenses of 7-day-old White Leghorn chickens were treated (15 mins) with 10  $\mu$ M of either a vehicle (dimethyl sulfoxide) or an inhibitor (1-(5-iodonaphthalene-1-sulfonyl)-1H-hexahydro-1,4-diazepine hydrochloride [ML-7], a myosin light chain kinase inhibitor; 1-phenyl-1,2,3,4-tetrahydro-4-hydroxypyrrrolo[2,3-b]-7-methylquinolin-4-one [blebbistatin], a myosin II inhibitor; latrunculin, an actin inhibitor). After treatment, lenses were incubated in antibodies against the appropriate cytoskeletal proteins, and mounted onto slides *in toto* for examination under confocal microscopy. Images taken from the confocal microscope were analyzed using nearest neighbour analysis for irregularity.

**Results** Myosin distributions were highly regular, with a nearest neighbour value ( $R_n$ ) of 2.02. Blebbistatin treatment resulted in a less regular arrangement ( $R_n=1.65$ ), approaching a random distribution. Actin distributions in the latrunculin-treated lenses were disorganized, showing irregular spatial distributions between vertices ( $R_n=1.53$ ) compared to those in vehicle treated lenses ( $R_n=1.91$ ).

**Conclusion** Inhibitors were shown to affect the distributions of actin and myosin, which indicates that these inhibitors impart their effects by altering cytoskeletal ultrastructure.

• S078

**Absence of beta-amyloid in cortical cataracts of donors with and without Alzheimer's disease**

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**Purpose** Eye lenses from human donors with and without Alzheimer's disease (AD) were studied to evaluate the presence of amyloid in cortical cataract.

**Methods** We obtained 39 lenses from 21 postmortem donors with AD and 15 lenses from age-matched controls. For 17 donors, AD was clinically diagnosed by general physicians and for 4 donors the AD diagnosis was neuropathologically confirmed. As controls, 7 donors with pronounced cortical opacities and 8 donors with almost transparent lenses were selected. All lenses were photographed in a dark field stereomicroscope. Histological sections were analyzed using a standard and a more sensitive Congo red protocol, thioflavin staining and beta-amyloid immunohistochemistry.

**Results** Of the 21 donors with AD, 6 had pronounced bilateral cortical lens opacities and 15 only minor or no cortical opacities. Thioflavin, standard and modified Congo red staining were positive in the control brain tissues and in the dystrophic cornea. Beta-amyloid immunohistochemistry was positive in the brain tissues but not in the cornea sample. Lenses from control and AD donors were, without exception, negative after Congo red, thioflavin, and beta-amyloid immunohistochemical staining.

**Conclusion** The absence of staining in AD and control lenses with the techniques employed lead us to conclude that there is no beta-amyloid in lenses from donors with AD or in control cortical cataracts.

• S079

**Absence of amyloid beta in human lens opacities: A confocal Raman study**

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**Purpose** Amyloid beta, the main protein component of Alzheimer (AD) plaques and tangles, is characterized by high levels of beta-sheets. Raman microspectroscopy allows quantitative analysis of this specific molecular conformation. We compared the beta-sheet levels in lens opacities and in plaques and tangles in the hippocampus of AD patients.

**Methods** We obtained 14 lenses from 7 post-mortem donors, neuropathologically confirmed as having advanced or moderate AD. From 3 of these donors, we also obtained hippocampus tissue. Protein and lipid conformations were analysed in the 500-1800 cm<sup>-1</sup> fingerprint region. The ratio of the beta-sheet peak at 1668 cm<sup>-1</sup> and the protein peak at 1450 cm<sup>-1</sup> is a quantitative measure for the beta-sheet content. Additionally, histological sections were stained using a standard Congo red protocol and amyloid beta immunohistochemistry.

**Results** The 1668cm<sup>-1</sup>/1450cm<sup>-1</sup> ratio, quantitatively reflecting the beta-sheet content, is 1.28 for clear and cataractous regions in the lens. For the plaques and tangles in the hippocampus the ratio is 1.62 and 1.23 for non-affected regions. When corrected for the presence of lipids in plaques and tangles this ratio is 2.64. Congo red and amyloid beta immunohistochemistry is positive for AD plaques and tangles but is negative for all lenses studied.

**Conclusion** In contrast with a previous study (Goldstein et al. 2003) we conclude that proteins in opaque regions of lenses and in hippocampal plaques and tangles are fully different species. Moreover opacification is not accompanied by changes in the beta-sheet configuration of lens proteins. This means that cortical cataract cannot be considered as an indicator and predictor of AD.

• S080

**Elastic properties of human lens zonules as a function of age in presbyopes**

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**Purpose** Ex vivo measurement of the elastic properties of human lens zonules as a function of age in presbyopes.

**Methods** We studied 16 presbyopic human donor eyes (ages 47 to 97). Anterior eye sections with crystalline lens, zonules, ciliary body and sclera were radially stretched. The stretching device consists of a chamber filled with balanced salt solution and eight radial hooks to hold the anterior eye section. Radial stretching was created with a stepper motor connected to a digital outside micrometer for linear displacement and digital balance for force measurement. Three eye globes were used to test our methodology. For 13 eye globes, the spring constant, elastic modulus of the zonular system, and Young's modulus of the zonules were calculated.

**Results** We found linear dependence for force-elongation and force-strain relationships at all ages. In young presbyopic eyes (ages 47 to 60), the Young's modulus of the zonules was 340 mN/mm<sup>2</sup>; whereas in older eyes (ages 83 to 97), it was significantly lower at 270 mN/mm<sup>2</sup>. However, the correlation coefficient between Young's modulus and age (47 to 97 years) was not significant with p = 0.063.

**Conclusion** The zonular system in presbyopic eyes is linear elastic, and the Young's modulus of the zonules decreases 20% from presbyopic age to late presbyopic age. However, there was no significant correlation between Young's modulus and age in presbyopes.

• S081

**Comparison of different techniques to evaluate the biomechanics of the accommodative apparatus in human eyes**

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**Purpose** For the study of the biomechanical properties of lens and zonules in a stretching device, we wanted to investigate the effect of keeping the ciliary body intact or cutting it radially.

**Methods** We studied the anterior eye segment of 3 pairs of presbyopic human donor eyes (ages 51-85). These segments, comprising of lens, zonules, ciliary body and sclera, were radially stretched in our device which allows force and tissue elongation measurements to be made. First, each pair (OD and OS) was stretched with the ciliary body intact and the lens in place, afterwards, in OD, the ciliary body was cut radially and the lens was kept, and in OS, the ciliary body was kept intact and the lens removed, then we stretched again. Forces are given at 500 µm stretch, measured from lens centre to ciliary body edge.

**Results** The mean force with the ciliary body intact and the lens in place was 74 mN. When the ciliary body was cut (with lens in place) the required force was 35 mN. When the ciliary body was kept intact and the lens removed we measured a force of 47 mN, which represents the contribution of the circumferential stretch of the ciliary body.

**Conclusion** Stretching anterior eye segments showed that the required forces were considerably higher when the ciliary body was kept intact as compared when it was cut radially. These forces were increased due to the circumferential resistance by the ciliary body.

• S082

**Retinal straylight and the yellow Bag in the Lens IOL**

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**Purpose** To evaluate the influence of blue-blocking Bag in the Lens (BIL) intraocular lens (IOL) on retinal straylight.

**Methods** Twenty-one patients scheduled for bilateral senile cataract surgery with a BIL intraocular implant (Morcher GmbH, Germany) randomly received implantation of either a UV light-filtering and blue-light filtering IOL (Morcher 89A yellow BIL) or a UV-light filtering IOL (Morcher 89A, clear BIL) in both eyes. Only the left eyes were included in this study. Best corrected visual acuity and retinal straylight level were evaluated 3 months postoperatively with decimal charts at 4m and with C-Quant (Oculus Optigeräte, Wetzlar, Germany) respectively.

**Results** There were 11 eyes in the yellow BIL group and 10 eyes in the clear BIL group. Preoperative measurements (K1, K2, anterior chamber depth, axial length, IOL power) showed no significant differences between the yellow and the clear BIL group. No significant difference in visual acuity was found between both groups ( $p = 0.512$ ). Mean retinal straylight did not differ significantly ( $p = 0.809$ ) between yellow BIL ( $1.24 \pm 0.26$ ) and clear BIL ( $1.19 \pm 0.21$ ), even after correction for age and axial length with the pseudophakic model ( $p = 0.557$ ).

**Conclusion** The UV and blue-blocking bag-in-the-lens had no influence on visual acuity and retinal straylight measured 3 months postoperatively, in comparison with a UV filtering bag-in-the-lens.

• S083 / 4458

**Halo size after implantation of a multifocal intraocular lens**

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**Purpose** To determine the size of a halo in the visual field induced by bright light after cataract surgery and implantation of an aspheric apodized diffractive multifocal intraocular lens (IOL) or aspheric monofocal IOL.

**Methods** This prospective observational study comprised 54 healthy eyes (mean age  $72.6 \pm 10.4$ ) of 54 subjects having cataract surgery with bilateral implantation of an AcrySof ReSTOR SN6AD1 IOL (32 eyes, multifocal group) or AcrySof SN60WF IOL (22 eyes, monofocal group). Six months postoperatively, halo size was measured using the Vision monitor. Optotypes of low luminance ( $5\text{cd}/\text{m}^2$ ) were presented at a distance of 2.5 m. The visual angle subtended by the radius of the halo was calculated in minutes of arc (arc min). Best-corrected distance logMAR visual acuity (VA) was evaluated with high-contrast (HC-96%) and low-contrast (LC-10%) logMAR Bailey-Lovie letter charts.

**Results** The mean radius of the halo was  $219.3 \pm 42.1$  and  $194.0 \pm 55.3$  arc min ( $p > 0.05$ ) in the multifocal and the monofocal groups respectively. The HC and LC VA means showed significant differences between the two groups, being worse in the multifocal group. Also, the HC and LC-VA exhibited a significant correlation with halo size ( $r = 0.52$  y  $r = 0.57$  respectively).

**Conclusion** Mean halo size was not significantly different between the multifocal ReSTOR SN6AD1 and the monofocal AcrySof SN60WF IOLs. Worse HC and LC-VA values were related to higher halo size measurements in the IOLs groups.

• S084

**Do multifocal intraocular lens implants allow spectacle independence?**

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**Purpose** This study aims to evaluate the effectiveness of multifocal and toric multifocal intraocular lens implants in achieving spectacle independence when used in cataract surgery and clear lens extractions.

**Methods** Case records were examined retrospectively for 218 eyes of 109 consecutive patients (38 male) who underwent lens extraction with multifocal intraocular lens implant by a single surgeon. If corneal astigmatism was present appropriate corrective procedures were undertaken: this included 38 eyes which received a toric multifocal lens. Data collected included: demographics, pre-operative corrected visual acuity (with habitual correction), post-operative unaided visual acuity for near and distance, degree of spectacle independence, and complications of surgery.

**Results** Over 90% of patients reported spectacle independence for daily activities. Complication rates were low, with tolerable glare and haloes being the most common problem. Approximately half required laser capsulotomy for posterior capsule opacification.

**Conclusion** In conclusion, multifocal intraocular lens implants can allow carefully selected patients to achieve spectacle independence. These results compare favourably with published data.

• S085

**Quality of vision with traditional monovision versus modified monovision**

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**Purpose** To compare binocular subjective quality of vision with modified monovision (MMV) (ie different combinations of spherical (SA4) and secondary spherical (SA6) aberration on each eye) versus traditional monovision (TMV) with different level of anisometropia

**Methods** A numerical eye model was used to generate images (ie three 0.4 logMAR high contrast letters) viewed through a 4.7mm pupil, degraded by various condition of SA and for proximities from -5D to 5D (Naked eye, SA4-0.4µm, SA4+0.4µm, SA4-0.4 and SA6+0.2µm, SA4+0.4 and SA6-0.2µm). Binocular vision was simulated using a 3D-NVIDIA video device projecting different image on each eye. Binocular through-focus (TF) quality of vision was evaluated using a grading scale (ITU-R 500 recommendation) by four subjects with TMV for different level of anisometropia (ie 0.5 to 3D at 0.5D step) and with various combination of MMV

**Results** Area under TF curve (ie a way to evaluate quality of vision) measured for a fair or higher image quality was increased with TMV and MMV compared to naked eye because of the summation of the two best monocular curves. With TMV, a 2.5D anisometropia was the more effective to increase area under TF curve with however a lack of intermediate vision. With MMV the greatest increase of quality of vision was obtained with reverse profile of SA4 and SA6 on each eye (ie SA4-0.4 and SA6+0.2µm on one eye and SA4+0.4 and SA6-0.2µm on the other eye). Compared to TMV, reverse profiles of MMV showed a significant benefit in improving area under TF curve thanks to a better quality of intermediate vision

**Conclusion** MMV especially with reverse profiles of SA improved overall quality of vision without the lack of intermediate quality of vision intermediate quality of vision induced by TMV

• S086 / 2643

**A novel imaging approach to periocular basal cell carcinoma: In vivo optical coherence tomography and histological correlates**

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**Purpose** Optical coherence tomography (OCT) is a non-invasive imaging method widely used in ophthalmology. Recent developments have produced OCT devices for imaging the skin. The purpose of this study was to assess preoperative OCT of periocular basal cell carcinoma (BCC) and to correlate optical biopsies with histological features.

**Methods** Consecutive patients with periocular BCC were prospectively investigated with VivoSight OCT (Michelson Ltd) prior to surgical excision. OCT images were correlated to haematoxylin and eosin stained histology sections with regard to: epidermal changes, intralesional and perilesional features.

**Results** A total of 15 patients with periocular BCC were recruited. Epidermal changes over BCC lesions represented important landmarks for topographic correlation and consisted in epidermal thinning and hyper-reflective signals from surface ulceration and crusting. Intralesional OCT features of BCC included: hyporeflective lobular patterns corresponding to BCC nests with high cellularity, hyporeflective cystic spaces of liquefactive necrosis and hyper-reflective margins corresponding to collagen compression. Perilesional OCT features of BCC included: hyporeflective spaces corresponding to fluid clefts, hyper-reflective bands of perilesional granulomatous inflammation secondary to tissue biopsy and hyporeflective lumens of blood vessels and hair follicles

**Conclusion** This study demonstrated a high correlation between OCT images and histological features. Further studies are necessary to investigate novel potential applications of in vivo OCT for BCC such as non-invasive diagnosis, intraoperative OCT-guided tumour excision and postoperative detection of recurrences.

• S087 / 2645

**Strabismus and diplopia revealing rhabdomyosarcoma in a 7-year-old girl**

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**Purpose** This case report presents the diagnosis and management of seven-years-old girl with persistent diplopia revealing orbital rhabdomyosarcoma ,a rare and malignant type of tumor among children. Few cases have been reported.The clinical presentation, complementary exams, and treatment for this condition are reviewed.

**Methods** A 7-year-old girl was referred to our department for persistent diplopia and unilateral exophthalmia on her left eye that had been lasting for 5 months.The orthoptic examination showed no visual acuity loss, vicious head tilt bent on the left side, exotropia with quick restitution, limitation of abduction and elevation on left eye. The Lancaster test confirmed the limitation.The eye fundus examination revealed signs of extrinsic compression.

**Results** Magnetic resonance imaging, and ocular and orbital echography showed an intra conical tumor of 18x21 millimeters, displacing the ocular nerve and invading the superior rectus muscle. Tumor biopsy revealed undifferentiated rhabdomyosarcoma. Metastatic work-up was negative and included thoracic and abdominal CT, bone scintigraphy and bone marrow biopsy. The treatment consisted in chemotherapy and permitted tumor volume regression of 85%. The patient has successfully managed with muscle surgery, not on the cicatricial site but on the other eye.

**Conclusion** Ocular rhabdomyosarcoma is a rare tumor among children, but the most frequent malignant orbital tumor, with possible secondary localisation. Early diagnosis and early treatment are determinant for the prognosis and the survival rate of these patients. This case report shows all the utility of strabismological examination to detect the first signs of the condition, even without any symptoms relative to visual acuity.

• S088 / 2644

**Unique morphology of the human orbit among the Hominoidea**

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**Purpose** Like many mammal predators, Humans have frontal (forward-facing) orbits. This design allows a large overlap of monocular visual fields with good stereoscopic vision but is considered to harm lateral space perception. In Humans, on average, temporal visual field extends 95° in primary position of gaze but 128° with eye abduction. Which anatomical peculiarity may allow such a visual field expansion?

**Methods** Comparative orbit osteology study in 100 human skulls and 120 Apes' skulls (30 gibbons; 30 orang-utans; 30 gorillas; 30 chimpanzees and bonobos). Orbit width and height were recorded. Using a protractor and laser levels two orbit angles were recorded: "convergence angle" (the lower this angle, the more frontal the inner orbital rim orientation) and "opening angle" (the higher this angle, the more backward the temporal orbital rim position).

**Results** The largest orbit width/height ratio is 1.19, in Humans ( $p < 0.001$ ). Humans have a higher "convergence angle" (98.1°) than all Apes except gibbons (99.2°;  $p > 0.05$ ). Humans have by far the largest "opening angle" (107.1°;  $p < 0.001$ ) and the largest difference between "opening angle" and "convergence angle" (9°;  $p < 0.001$ ).

**Conclusion** The largest orbit width/height ratio found in Humans suggests a design that favours lateral vision. More specifically, human orbital rim is unique in that, while frontal, it has by far the most backward temporal orbital rim. This peculiarity - likely and adaptation to terrestrial life with upright bipedal locomotion - allows both good stereoscopic vision and large temporal visual field extent through eye motion.

• S089 / 1777

**Orbital melanocytosis and OTA naevus**

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**Purpose** Presentation and discussion of a rarely reported location of orbital melanocytosis

**Methods** Case report

**Results** In a 54 year old man with an oculodermal melanocytosis (naevus of OTA), enucleation was performed for a choroidal melanoma. During the surgery spots of hyperpigmentation, based on melanocytosis, were found in tendons, muscles and orbital fat. Oculodermal melanocytosis is classically described on the periocular skin, sclera, uvea, orbit, meninges, palate or tympanic membrane and is a well-known risk factor to develop choroidal and orbital melanoma. Few reports describe the particular involvement of the orbital tissue. We show the pictures of orbital tissue and extraocular muscle pigmentation and explain it with embryology.

**Conclusion** Even after enucleation for a choroidal melanoma in an OTA naevus, the ophthalmologist should be alert that extraocular melanocytes can remain and potentially be the cause of an orbital melanoma.

• S090

**Different assessments of immunohistochemically stained Ki-67 ocular malignancies**

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**Purpose** To investigate a tumor proliferation marker Ki-67 in conjunctival malignancies and to compare the results of different quantitative assessments.

**Methods** This study was designed as a pilot study to assess nuclear proliferation protein (Ki 67) in conjunctival malignancies. Immunohistochemical staining of Ki-67 was evaluated in 8 surgical samples of ocular surface neoplasias: 6 cases of invasive conjunctival squamous carcinoma and 2 of conjunctival melanoma. Ki-67 counting and evaluation was assessed on selected areas using different methods: manual counting (MC) of >1500 cells; digital image analysis with Nuclear V9 algorithm and digital image analysis using stereology module for ImageScope of Aperio. Ki-67 values from manual, stereoscopic and automated analyses were compared.

**Results** The MC, the nuclear V9 algorithm analysis and stereologic method revealed positivity of Ki-67 in invasive conjunctival squamous carcinoma from 22 to 60%, 21.5 to 43.5 % and from 30.1 to 51.5% respectively; in conjunctival melanomas from 20 to 30 %; from 17.8 to 18%, and from 7.17 to 29.1% respectively. The strongest correlation was observed between Aperio algorithm and stereologic method in conjunctival squamous carcinoma (0.87). Weaker correlation was found between Aperio/manual (0.6) and manual/stereological (0.83) methods.

**Conclusion** Our data demonstrate that different methods of Ki-67 evaluation may give dissimilar results, but results of this computer assisted nuclear V9 algorithm correlates strongly with stereoscopic analysis and can be helpful in accurate quantitative evaluation of immunohistochemically stained biomarkers.

• S091

**Rare iridociliary pediatric tumours and pseudotumors: Cases report and literature review**

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**Purpose** Iridociliary tumours are usually uncommon in childhood. Our aim is to describe cases of juvenile xanthogranuloma, iris tuberculoma and medulloepithelioma in young patients and to differentiate them from other similar lesions

**Methods** Retrospective cases study. Four patients ( two girls and two boys) were referred at the Referral Center for Retinoblastoma of the University of Siena. They underwent all the ophthalmological examination including standard procedures, MRI of the orbits, CT, UBM, immunological and molecular analysis. In three cases organ-preserving operations were performed.

**Results** Two tumors were histologically identified as medulloepithelioma. Two tumor-like lesions were determined as juvenile xanthogranuloma and iris tuberculous granuloma. In the last case, antituberculous therapy was performed

**Conclusion** Iridociliary tumors and pseudotumors are rather rare in childhood. Nevertheless, they should be taken in consideration in differential diagnosis with other pediatric intraocular tumors, particularly retinoblastoma, ciliary body adenoma and adenocarcinoma. An executive checkup including pathology, immunohistochemistry and immunology is to be performed.

• S092

**Expression of stem cell markers in human uveal melanoma**

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**Purpose** Uveal melanoma is a rare but very aggressive malignancy. Independently of the currently available therapies, 50 % of uveal melanoma patients will develop metastasis and the average survival time of these patients is only 2-8 months. Genetic and epigenetic background of uveal melanoma is not fully understood and useful prognostic markers for metastasis development are not well characterized. It was suggested that in various tumors cancer stem cells are responsible for the development of primary tumors and their metastases. In uveal melanoma, the role of cancer stem cells is not clear that's why our aim with the present study was to investigate the expression of mRNA of stem cell markers.

**Methods** Human uveal melanoma specimens were obtained from 31 enucleated patients at the University of Debrecen, Department of Ophthalmology. The expression of stem cell markers was studied by RT-PCR, using gene specific primers for FZD6, NES, NGFR, PROM1 and SOX10.

**Results** mRNA for FZD6 and SOX10 could be detected in 100% of the investigated samples. NES, NGFR and PROM1 were present in 94%, 94% and 82%, respectively.

**Conclusion** Results of our study demonstrate that certain stem cell markers are present in nearly all uveal melanoma specimens. Therefore, they are not suitable as prognostic markers. However, our findings that a high percentage of uveal melanomas express stem cell markers support the view that stem cell genes may play an important role in the development and progression of this aggressive cancer. Further studies are needed to evaluate the exact role of these markers and to explore potential therapeutic approaches to target cancer stem cells in human uveal melanoma. Grant support: TÁMOP 4.2.2.A-11/1/KONV-2012-0025 (G.H.)

• S093

**Metastases inhibition and cellular damage in melanoma cells irradiated with proton beam**

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**Purpose** Proton beam therapy has been used in clinic with a great success, however, not many experimental studies have been performed. The goal of our study was to determine the cellular response to low, sublethal doses of proton beam irradiation, in particular DNA damage, cell cycle arrest, changes in expression of proteins, and effect on metastases in vivo.

**Methods** BLM cells were irradiated with 1-7 Gy of proton beam irradiation. The source of the 58 MeV proton beam was the AIC-144 cyclotron at Institute of Nuclear Physics, Polish Academy of Sciences, Kraków. Tumors of Bomirski Hamster Melanoma (BHM) implanted into the anterior chamber of the hamster eye grew aggressively and completely filled the anterior chamber within 8-10 days. Metastases, mainly in the lung, were found in 100% of untreated animals 30 days after enucleation. The protons were accelerated using AIC-144 isochronous cyclotron, operating at 60 MeV and BHM tumors located in the anterior chamber of the eye were irradiated with 10 Gy, for the depth of 3.88 mm.

**Results** Slow accumulation of damage was observed reflected in slowing of the proliferation rate, and increase in caspase activity with time. The number of cells in G2/M and >2n increased with proton beam dose. Proton beam irradiation caused upregulation of proteins involved in: DNA repair, RNA functioning (i.e. stress granule and P-bodies components), apoptosis and survival processes and downregulation of enzymes engaged in glycolysis. Of particular interest was heavy downregulation of vimentin (2.4 times), involved in structural integrity of cells and tissues, adhesion and migration, and other processes. Irradiation led to changes in cell migratory properties. Proton beam irradiation caused inhibition of tumor growth by about 10 days and inhibition of metastatic spread.

• S095

**Photodynamic therapy for juxtapapillary retinal capillary hemangioma: A case report**

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**Purpose** Various treatment modalities have been described for retinal capillary hemangioma therapy, including observation, laser photocoagulation, cryotherapy, radiotherapy and vitreoretinal surgery. Our purpose is to present a case of juxtapapillary retinal capillary hemangioma treated with photodynamic therapy (PDT).

**Methods** A 69-year-old woman with no previous ocular history presented with blurred vision and photopsias in the right eye since three months. The patient underwent a thorough ophthalmological examination, including visual acuity measurement by means of Snellen charts, slit-lamp examination, intraocular pressure (IOP) measurement and dilated fundoscopy.

**Results** At presentation, her best corrected visual acuity (BCVA) was 6/9 in the right eye and 6/6 in the left eye. The anterior segment was totally normal and IOP was normal in both eyes as well. Dilated fundoscopy revealed a yellowish, elevated area with blood vessels, on the inferior margin of the right optic disc, as optic disc edema. Fluorescein angiography and angiogram with indocyanin green confirmed the diagnosis of juxtapapillary retinal capillary hemangioma. The patient was treated with PDT with verteporfin and three months later her visual acuity was 6/7.5 in the right eye and the lesion was slightly smaller.

**Conclusion** Retinal capillary hemangioma should be included in the differential diagnosis of optic disc edema. PDT offers promising results as far as anatomical and functional results, providing visual acuity improvement or even stabilization and restriction of enlargement of the lesion.

• S094

**Trans-scleral local resection of toxic choroidal melanoma after proton beam radiotherapy**

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**Purpose** To report on trans-scleral local resection of choroidal melanoma as treatment for serous retinal detachment after proton beam radiotherapy (PBR)

**Methods** Retrospective study of patients underwent trans-scleral local resection of choroidal melanoma after previous PBR from 2000 to 2008 to the Liverpool Ocular Oncology Service.

**Results** The 11 patients (5 female, 6 male) had a median age of 48.8 years (range 20 – 76) included in this study. The tumor margins extended anterior to ora serrata in 6 patients. On echography, the largest basal tumor dimension averaged 11.9 mm (range 5.7 – 13.2) and the tumor height averaged 7 mm (range 4.5 – 9.9). The retinal detachment was total in 4 cases. Neovascular glaucoma was present in 4 patients. The time between proton beam radiotherapy and local resection had a mean of 17.4 months (range 1 – 84). The follow-up time after the local resection procedure had a mean of 96 months (range 60 – 156). At the last known status, the eye was conserved in 9 patients, with a flat retina in all of these patients and visual acuity better than 6/30 in 5 patients. The reasons for enucleation were: retinal detachment (1 patient) and phthisis (1 patient).

**Conclusion** Serous retinal detachment and neovascular glaucoma after proton beam radiotherapy of a choroidal melanoma can both resolve after trans-scleral local resection of the tumor. Our findings indicate that these complications are caused by the persistence of the irradiated tumor within the eye ('toxic tumor syndrome').

• S096

**Cases of choroidal metastasis revealing lung cancer**

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**Purpose** Choroidal metastases are the most common intraocular malignant tumor but rarely reveal cancer. However the role of the ophthalmologist is central to diagnose this disease. Ophthalmological examination, optical coherence tomography, ultrasound and angiography may help for differentiate metastasis to other choroidal tumors. The aim to this presentation is to describe to identical cases of choroidal metastasis revealing lung cancer.

**Methods** We report 2 cases, a 53-year-old woman and a 63-year-old man, with no prior history of neoplasia who consulted for decreased visual acuity of the right eye. In both, eye fundus examination reveals a suspicious yellowish choroidal lesion. Fundus examination, optical coherence tomography (confirming the serous retinal detachment), ultrasound (confirming the plan or thick appearance) and angiography (showing blocking defect in early stage and hyperfluorescence with pin points in late stage for fluorescein angiography and diffuse hypofluorescence in late stage for indocyanin angiography) led us to the conclusion of choroidal metastasis. Then, next investigations were mammography (for woman) and body scan (for both). The results reveal an adenocarcinoma of the lung for both cases.

**Conclusion** Choroidal metastases are rarely inaugural of a cancer but an early diagnosis is essential. The role of ophthalmologist is central to the diagnosis and both cases allow remembering the main diagnostic criteria.

• S097

**Synchronous malignant transformation of bilateral symmetrical retinocytoma**

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**Purpose** To report the outcome of a 4-year-old girl who was found to have bilateral malignant transformed retinocytoma.

**Methods** The patient was treated with systemic chemotherapy and photocoagulation followed by intraarterial chemotherapy.

**Results** Complete remission was achieved.

**Conclusion** Chemotherapy destroyed malignant retinoblastoma and the "retinoma" component remained unchanged.

• S098

**Case of primary neuroendocrine carcinoma in lacrimal gland**

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**Purpose** Case of primary neuroendocrine carcinoma in lacrimal gland

**Methods** A 86-year-old man noticed a swelling of the left upper eyelid three month earlier. Because the size of the nodule gradually increased, the patient was referred to the Chiba University Hospital. We performed excision biopsy and histopathological examination indicated that the tissue had low-grade atypical cells which were positive to CD56, CK7, and CKAE1/AE3. Magnetic resonance imaging and positron emission tomography excluded systemic metastasis. The patient was diagnosed with a primary NEC of the left lacrimal gland.

**Results** He underwent chemotherapy (Carboplatin and etoposide) for four cycles, and the size of the tumor was significantly decreased. A month later, we performed tumor excision including the clinically visible margin, and a month later the patient had 50Gy radiotherapy at the surgical region. After the three-combination therapy, the tumor was undetectable in the MRI. A month later, however, a metastasis to the parotid lymph node was found, and the patient underwent total lymphadenectomy of the left parotid gland and 46Gy radiotherapy was applied to the left neck. At the last examination, the patient had neither recurrence nor metastasis.

**Conclusion** Although primary NEC of the lacrimal gland is extremely rare, the tumor has high malignancy and readily metastasizes. Thus, combined surgery, radiotherapy, and chemotherapy are needed for the complete management of NEC.

• S099

**Mucormycosis is a rare etiology of ophthalmoplegia:  
How to diagnose and to manage it, about one exceptional case**

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**Purpose** Mucormycosis is a rare opportunist infection characterized by a high mortality rate occurring in immunodeficient patient population. Its rhino-cerebral form may be complicated by neuro-ophthalmological and orbital symptomatology.

**Methods** We report a case of a 57 years old woman, referring because of diplopia associated with a diabetic ketoacidosis. Ocular examination revealed a complete left ophthalmoplegia. Magnetic resonance imaging highlighted a cavernous sinus thrombosis associated with ipsilateral pansinusitis. In this context, mucormycosis diagnosis is discussed and confirmed by anatomopathology analysis after sinus biopsy. The follow-up is exceptional in its ophthalmological complications despite antifungal agents and anticoagulant therapy. We observed consecutively a left central retinal artery occlusion (CRAO) then a contralateral central retinal venous occlusion, and finally a left neovascular glaucoma.

**Results** Mucormycosis is a fungal opportunist infection that must be very early diagnosed. This pathology is characterized by sinus infiltration, necrosis, and bony walls damage. Medical treatment by anti-fungal agents alone, can not contain it. It must be associated with a wide and repeated surgical debridement of necrotic tissue. Symptomatic ocular treatment (anti-VEGF, Laser) is needed in order to preserve visual function and anatomical prognosis. Indeed, the extension along blood vessels walls leads to thrombosis and necrosis, that is resistant to systemic therapy. Our patient is still alive after a follow-up of 24 months.

**Conclusion** This case illustrates a serious multidisciplinary pathology that is very difficult to control. The ocular signs were predominant but revealed a large rhino-cerebral infiltration.

• S100

**A time-line model of developmental events within the human fovea based on imaging and histology data**

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**Purpose** To create a time-line model of developmental events during normal maturation of the inner and outer foveal layers based on imaging and histological studies.

**Methods** Data from recently published imaging studies using hand-held OCT during the period following premature birth (Dubis et al., 2012; Maldonado et al., 2012; Vajkovic et al., 2012) and histology of normal donor eyes (Dubis et al., 2012; Hendrickson et al., 2012; Vajkovic et al., 2012) was used to establish the timing of foveal developmental events from the time of mid-gestation to early childhood. A model of the onset and maturation of the following foveal developmental events was constructed: Pit formation, extrusion of inner retinal layers, widening of the outer nuclear layer, separation of the hyperreflective inner segment layer from the RPE, and lengthening of the outer segment. Developmental events were graded as incipient, intermediate or mature.

**Results** The normal developmental events within the central fovea are depicted along a logarithmic time-line. The developmental models based on OCT or histology of the inner and outer retina show a close correspondence. The structural elements of the inner retina all reach near full to full maturity at term birth. At this time the outer retinal structures are immature and the maturation of the photoreceptor layers starts at an age of some postnatal weeks and reach near full to full maturity at one to three years of age.

**Conclusion** The inner and outer foveal layers reach maturity within different time frames; the inner layers during the late part of gestation, and the outer layers during the first postnatal years. Our model is of importance when evaluating abnormalities in e.g. prematurity or albinism.



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