SIRCOVA-OFTARED Congress Abstracts
Valencia, June 6–8, 2013

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About SIRCOVA
SIRCOVA, the Society for Research in Retina was formed in 2009 as a result of collaborative efforts by a wide spectrum of professionals interested in ophthalmology and visual sciences research, that have given up their own identities, and all together joined this new society.

SIRCOVA was born from a multi-disciplinary viewpoint, with the challenge of encouraging investigation, innovation and the development of biotechnology in retina and visual sciences.

SIRCOVA is a non-profit organisation with the main goal of the dissemination of knowledge concerning the retina, and the visual sciences, by means of meetings, publications, professional development and education courses, workshops and laboratory updating events and exchange activities. Because of this, SIRCOVA is open to any interested in these topics. Membership forms and a copy of the most relevant constitution documents can be obtained from the secretariat or via internet. Any group within-or-out SIRCOVA, may propose and organise symposia and other related activities under the board of directors supervision. This year the Spanish Macula Club has joined our Congress for their workshop. The 2013 SIRCOVA congress has been officially recognized as an activity of the Ocular Pathology National Net (OFTARED) of the Institute of Health Carlos III (Spanish Government).

SIRCOVA congress will be held every 18 months (in June or in November of the corresponding year).
Secretariat SIRCOVA: Ilustre Colegio de Medicos de Valencia, Av/ de la Plata 20; 46013 Valencia, Spain. info@sircova.es www.sircova.es.
As a foundational member of the Society of Research in Vision and Ophthalmology (SIRCOVA), it is my pleasure to welcome you all to Valencia for our 3rd Congress. The SIRCOVA Organizing Committee led by Prof. Manuel Vidal-Sanz and the Commissions has assembled an excellent scientific program to generate both knowledge and new idea sharing. Thank you to all of you for outstanding help.

Fundamental purpose of our Congress is to provide a forum at which a multi-disciplinary array of ophthalmologists, scientists, optometrists, students, health care professionals and industry representatives can learn, investigate and create ideas, the outcomes of which are clinical applications to better care ophthalmological patients.

I encourage all to take advantage of the six educational courses, five expert sessions, six plenary lectures, one symposium, six sections for free communications (express), and other activities that are fully described through the program (www.sircova.es).

The abstracts from the SIRCOVA 2013 Congress highlight the most relevant issues in the diagnosis, management and treatment of ocular disorders, ranging from basic to clinical and population research.

Thank you to the submitters who sent in their research. Selection of the adequacy of all submitted works was carried out by the Congress Commissions. The Congress Publication Committee was formed by the following: Prof. Neville Osborne, Prof. Nicolas Cuenca, Prof. M. Dolores Pinazo-Durán and Prof. Vicente Zanon-Moreno, Prof. Carlo Nucci (Basic Research); Dr. Amparo Navea, Prof. Maria Paz Villegas, Prof. Jose J. Garcia-Medina and Prof. Rosa de Hoz (Clinical Research); Prof. Manuel Diaz-Llopis and Dr. Roberto Gallego-Pinazo (Clinical Cases). Therefore, those authors selected for the outstanding quality of their work will be competing for a SIRCOVA Award. A panel of international experts will select a winner and two honor mentions for the oral communications (express sessions: 3 minutes/3 slides). Awards are focused on Basic Science, Clinical Science, Clinical Case and Doctoral Thesis.

The exciting combination of the dynamic scientific program and the variety of attractions of the charming cultural capital of VALENCIA are proving irresistible to many who have already decided to come to the 2013 SIRCOVA Congress!

Thank you for join us in Valencia for what promises to be a wonderful event.

*Maria Dolores Pinazo-Durán*
SIRCOVA President
Clinical Research

1  Erythropoietin in Eyes of Patients with Retinitis Pigmentosa  
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Purpose: To determine the levels of erythropoietin (EPO) in aqueous humor of patients with retinitis pigmentosa (RP).

Material and Methods: A prospective, comparative control study. Aqueous humor was collected from eyes of patients with RP. The level of EPO was determined with a commercially available enzyme-linked immunosorbent assay kit. The control group comprised aqueous samples from patients about to undergo cataract surgery and without any other ocular or systemic disease.

Results: The concentration of EPO in aqueous humor was markedly elevated in patients with RP than in control subjects (Mann-Whitney U test, P < 0.001). The level of EPO was 37.43 ± 16.15 (mean ± SD) mU/mL in eyes with RP and 16.88 ± 7.31 mU/mL in the eyes of the control group.

Conclusion: In patients with RP the concentration of EPO in aqueous humors is higher than in non-RP subjects. This finding may indicate a response to injury and provides new evidence that should be taken in account when considering EPO as a neuroprotective treatment for patients with RP.

2  Oxidative Stress Markers in the Aqueous Humour and Peripheral Blood of Patients with Retinitis Pigmentosa  
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Purpose: Retinitis Pigmentosa (RP) is a common form of hereditary retinal degeneration characterized by loss of retinal photoreceptor cells. It has been suggested that after rod death, cone die due to several causes including oxidative damage which probably plays a major role in the pathogenesis of RP. We evaluated the presence of oxidative stress markers in aqueous humour and peripheral blood of patients with RP and compared them with those found in healthy controls.

Material and Methods: We measured the levels of total antioxidant capacity (TAC), free nitrotyrosine, thiobarbituric acid reactive substances (TBARS) formation, superoxide dismutase (SOD) activity, protein, metabolites of the nitric oxide/cyclic GMP pathway, heme oxygenase-1 and inducible nitric oxide synthase expression in aqueous humour and peripheral blood from sixty-six RP patients and sixty subjects without systemic or ocular oxidative stress-related disease. Ophthalmic test included were best-corrected visual acuity and visual field.

Results: MANCOVA revealed that RP reduced ocular TAC including SOD activity and induced nitrosative-oxidative stress markers as TBARS or nitrotyrosine in blood.

Conclusions: Our study suggests that RP reduces antioxidant defense on eye and induces nitrosative-oxidative damage in blood.

3  Contrast Sensitivity in Patients with Alzheimer’s Dementia in Initial Stages  
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Purpose: Alzheimer’s disease (AD) is characterized by the appearance of neurofibrillary tangles and amyloid plaques in cerebral cortex, including several areas known to be involved in visual processing. Contrast sensitivity (CS) test allows the analysis of retinal ganglion cell function and their cortical processing. Our purpose was to analyze if CS is affected in early Alzheimer’s degenerative processes.

Material and Methods: Twenty early AD patients (GDS 3–4) and 25 controls were examined. AD patients were diagnosed at Hospital Clínico San Carlos in Madrid. Two AD patients were excluded for visual acuity less than 0.6 or retinal pathology. Patients underwent a complete ophthalmologic examination (visual acuity, refraction, CS, biomicroscopy, IOP, and dilated fundoscopy). The CS test used was CSV-100OE chart test. This test is presented at 2.5 meters. It provides for four rows of sine-wave gratings. These gratings test the spatial frequencies of 3, 6, 12 and 18 cycles/degree.

Results: CS was lower in AD patients than in control (p < 0.01) for all the spatial frequencies, the greatest decrease involving high frequencies.

Conclusions: CS is affected in early stages of AD. This test could be a useful tool for early AD diagnosis.
4

Involvement of USH2 Genes in Spanish Patients with Usher Syndrome
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Purpose: Usher syndrome type 2 (USH2) is an autosomal recessive disease characterized by moderate to severe hearing loss and retinitis pigmentosa. To date, three disease-causing genes have been identified, USH2A, GPR98, and DFNB31. The aim of this work was to determine the contribution of USH2 genes in a Spanish cohort of 88 patients using exhaustive molecular analysis.

Material and Methods: Linkage analysis was performed to prioritize the gene to study, followed by sequencing of exons and intron-exon boundaries of the selected gene, USH2A (72 exons), GPR98 (90 exons) or DFNB31 (12 exons). Functional splicing analyses and comparative genomic hybridization array to detect large rearrangements were performed when appropriate.

Results: Forty-three patients carried mutations in USH2A and seven in GPR98. No pathological mutations were identified in the DFNB31 gene. Mutations in these genes are of all types, including large gene rearrangements, are spread throughout the genes and mainly are private.

Conclusions: In Spain, USH2A and GPR98 are responsible for 95.8% and 5.2% of USH2 mutated cases, respectively. DFNB31 gene. Mutations in these genes are of all types, including large gene rearrangements, are spread throughout the genes and mainly are private.

5

Autologous Fibrin Membrane for the Management of Corneal Perforations
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Purpose: To report the use of Autologous Fibrin Membrane and E-PRP clot to seal corneal perforations related to different severe corneal ulcerative diseases.

Material and Methods: 11 patients with corneal perforation were treated. Platelet rich plasma clots were introduced onto the corneal perforation and the epithelial debrided area underneath the fibrin membrane. At the end of the procedure, a temporary partial tarsorrhaphy was performed. Minimum postoperative follow-up was 3 months.

Results: The autologous fibrin membrane obtained was circular with a diameter between 18–22 mm and a thickness of approximately 1 mm. With this shape and size the fibrin membrane was perfectly manageable and suitable for applying to the damaged ocular surface. After the procedure all cases of corneal perforation were sealed. No evidence of infection/inflammation was detected. No patients reported pain, discomfort or any subjective symptoms. After 3 months follow-up there was no evidence of relapses or perforations. Due to the severity of the corneal diseases corneal grafting was performed in 8 of the 10 cases.

Conclusions: The combined use of Autologous Fibrine Membrane and E-PRP clot can be considered as a safe and effective alternative for the closure of corneal perforations to the traditional amniotic membrane transplantation procedure.

6

Cytokines and Infectious Markers in Retinal Detachment and Proliferative Vitreoretinopathy
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Purpose: To evaluate pro-inflammatory cytokine expression in the vitreous (V), subretinal fluid (SRF) and plasma (PL) from patients with rhegmatogenous retinal detachment (RRD) with (C) or without (–C) proliferative vitreoretinopathy (PVR). Existence of infectious markers (herpes-virus and bacteria) was also tested for better managing RD.

Material and Methods: Cytokine expression was assayed by the ELISA “sandwich” technique in the V, SRF and PL from 27 samples (CG: 11 samples; RRD/PVR<C: 7 samples, RRD/PVR–C: 8 samples respectively).

Results: Only IL-6 showed significant differences [CG: 5.4 ± 4.3 pg/ml vs RRD/PVR<C: 49.0 ± 25.6 pg/ml and RRD/PVR–C: 396.1 ± 228.1 pg/ml (p < 0.05)]. PCR-RT results show no positive data for the HSV in all samples and only in two for the VZV. The PCR-RT results for Universal Pan-bacterial assays were positive in 27 samples (CG: 11 samples; RRD/PVR<C: 7 samples, RRD/PVR–C: 8 samples respectively).

Conclusions: Most relevant pro-inflammatory cytokine in patients with RRD/PVR<C was the IL-6. It was not established a relationship between infectious etiology and RRD or PVR initiation or progression. Data support the bacterial DNA positivity in the macular hole/epimacular membrane control patients suggesting infectious source in these pathologies.

7

Choroidal Thickness in Pathologic Myopia and Diabetic Retinopathy
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Purpose: To evaluate macular choroidal thickness (CT) in highly myopic eyes treated with PDT and/or anti-VEGF (IVR), and in eyes with different stages of diabetic retinopathy (DR), assessing the anti-VEGF effect.
Material and Methods: Forty-two highly myopic eyes were assigned to four groups: PDT, IVR, PDT + IVR and dry maculopathy (contralateral eyes without CNV); and 25 DR patients (50 eyes) to 2 groups: 1) NPDR + DME in both eyes (OU) treated with laser OU and anti-VEGF in one eye (n = 11); 2) PDR OU treated with PRP OU and anti-VEGF in one eye (n = 14). Images were acquired with SD-OCT (EDI protocol).

Results: In highly myopic eyes regression analysis showed that age (p < 0.001), axial length (p < 0.001), tessellated fundus (p = 0.046) and patchy atrophy (p = 0.008) were predictive of CT, but type of treatment was not. As for DR patients, eyes treated with anti-VEGF showed a reduction on macular CT comparing with eyes treated only with laser, in both groups (NPDR + DME, p = 0.04; PDR, p = 0.02). A negative correlation between the number of injections and CT was found (p = 0.03; R = –0.42).

Conclusions: Older age and greater axial length are major factors associated with choroidal thinning in treated highly myopic eyes. Diabetic eyes treated with anti-VEGF showed a reduction on CT.

8 Intravitreal Therapies in Exudative Atrophic Age-Related Macular Degeneration
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Purpose: To evaluate the efficacy of intravitreal pharmacotherapy in patients with non-exudative age-related macular degeneration (AMD) and subretinal and/or intraretinal fluid in the absence of choroidal neovascularization (CNV).

Material and Methods: Retrospective descriptive case series study of 25 eyes of 17 patients. Fluorescein angiography (FA) and indocyanine green angiography (ICGA) were performed at baseline to exclude CNV. All patients underwent best-corrected visual acuity (BCVA) measurement, dilated fundus examination (DFE) and spectral-domain optical coherence tomography (SD-OCT) at baseline and in every visit. Wilcoxon signed-rank test was performed for statistical analysis.

Results: In 18 treated eyes BCVA improved in 12 eyes (66.6%) with no significant differences (p = 0.11) and CST significantly decreased in 10 eyes (55.5%) from 289.5 ± 69.8 μm at baseline to 253.55 ± 70.12 μm (p = 0.04). We didn’t evidenced any case of conversion to neovascular AMD through a 83.80 weeks [range: 4–173 weeks] follow-up period.

Conclusions: Subretinal and/or intraretinal fluid may be present in cases of AMD even without signs of CNV. In such cases, intravitreal pharmacotherapy may improve the morphometric parameters without significant visual benefit, and on the other hand may prevent the progression to CNV. Further studies are warranted to confirm our preliminary data.

9 Intravitreal Ranibizumab for Diabetic Macular Edema
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Purpose: To assess the efficacy and safety of intravitreal injections of ranibizumab administered with pro re nata optical coherence tomography based therapeutic approach in patients with naïve DME.

Methods: After obtaining institutional review board approval the charts of all patients with DME diagnosed between October 2009 and May 2011 were reviewed. Inclusion criteria were: treatment-naive DME and at least 12 months of follow-up. Snellen best-corrected visual acuity (BCVA) and SD-OCT were collected at baseline and 12-months follow-up visits.

Results: Eighty eyes of 80 patients met the inclusion criteria (46 male, 34 female). The mean age was 78.5 years [range: 55–96]. BCVA improved from 0.37 ± 0.25 at baseline to 0.42 ± 0.25 (P = 0.044). The central subfield thickness decreased from 468.8 ± 196.8 at baseline to 368.8 ± 158.7 microns at 12-months follow-up (P < 0.001). The mean number of injections was 3.30 ± 1.5 per patient in 12-month follow-up. There were no local or systemic complications related to the injection.

Discussion: Our results suggest that intravitreal injections of ranibizumab were effective and safe in the treatment of DME. Although we report a monthly SD-OCT follow-up, the mean number of injections was 3.30 per patient in 12-month follow-up.

10 OCT Images Automated Labeler (OCTAL) in Different Macular Pathologies
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Purpose: To develop and assess the technical validity of new computer-aided diagnostic software (CAD) for automated analyses of optical coherence tomography (OCT) images for screening macular pathology.

Material and Methods: Technical validation was performed by a retrospective study design based on OCT images from clinical charts (normal, ERM, MH, RVO, and DME). Images were classified as normal or abnormal to serve for screening purposes. Sensitivity, specificity, positive predictive values and negative predictive values were obtained.

Results: The sensitivity of the CAD was 97.4% and the specificity 96.2%. Positive predictive value was 99.1% and negative predictive value was 89.3%.

Conclusions: This new CAD for automated analysis of OCT images offers adequate sensitivity and specificity to distinguish normal OCT images from those showing potential macular pa-
thology. These results will enable its clinical validation and a subsequent cost-effectiveness assessment to be made before recommendations are made for population-screening purposes.

11 Neuro-Ophthalmological Assessment Using Spinal Anaesthesia
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**Purpose:** The aim of the study was to compare the neuro-ophthalmological and anaesthetic effects of isobaric levobupivacaine and bupivacaine when intrathecally administered for knee arthroscopy.

**Material and Methods:** A prospective, double-blind, randomized study with 60 ASA grade I–II patients aged 18–65 years awaiting knee arthroscopy under spinal anaesthesia. There were two patient groups of 15 which received 12.5 mg of isobaric bupivacaine or levobupivacaine. Upon arrival at the anaesthetic room, and 5 minutes after initiating spinal anaesthesia, a complete ocular examination was performed, including the tests that follow in this order: 1) near best-corrected visual acuity of each eye; 2) Amsler Grid Test; 3) extrinsic and intrinsic ocular motility assessment; 4) Bielchowsky test. When surgery ended, each patient completed a neuro-ophthalmologic symptoms questionnaire. It also was completed by telephone at 24 hours, 72 hours and 1 week after spinal anaesthesia.

**Results:** No neuro-ophthalmological side effects were noted before or after spinal anaesthesia in either group. Follow-up on days 1, 3 and 7 post-surgery revealed neither neuro-ophthalmological symptoms nor other side effects.

**Conclusions:** Intradural anesthesia with bupivacaine or levobupivacaine have no perioperatory neuro-ophthalmological side effects.

12 Choroidal Thickness in Highly Myopic Eyes with/without Macular Retinoschisis
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**Purpose:** To measure macular choroidal thickness (CT) in highly myopic eyes (HME) and to correlate with macular retinoschisis using enhanced depth imaging optical coherence tomography (EDI-OCT).

**Methods:** This is a retrospective, observational case-series study. EDI-OCT images were obtained in (HME) (≥6 D), with/without retinoschisis, with a Heidelberg Spectralis OCT. Choroidal thickness was measured subfoveolar. Statistical analysis was performed to evaluate CT and to correlate it with age, refractive error and axial length on eyes with/without macular retinoschisis.

**Results:** We studied 41 eyes of 21 patients mean aged 50.2 ± 12.7. Mean refractive error was −12.2 ± 3.8 D. The axial length mean was 29.6 ± 2.4 mm. Fifteen eyes had macular retinoschisis (36.6%). The mean CT was 87.4 ± 76.9 μm. It was correlated negatively with the axial length (p < 0.001), age (p = 0.001) and refractive error (p = 0.002), but not with presence or absence of retinoschisis. Regression analysis suggested that subfoveal CT decreased 18 μm for each mm of axial length.

**Conclusions:** Choroidal thickness was reduced in HME and correlated indirectly to the degree of myopia. Macular schisis seems to be an independent finding in our series. This result provides additional evidence that macular schisis is not related with choroidal changes and support the vitreous tractional theories.

13 Changes in Optical Quality after Cataract Surgery
Reina Sofia Hospital, Córdoba, Spain

**Purpose:** To assess optical quality changes after phacoemulsification surgery combined with the implantation of Akreos Adapt M160 IOL.

**Material and Methods:** This prospective study included 30 eyes, in which cataract surgery with intraocular lens implantation was performed. The lens was Akreos Adapt M160 IOL. Optical quality was analyzed using the Optical Quality Analysis System (OQAS II, Visiometrics, SL). Objective scattering index (OSI), modulation transfer function (MTF) and accommodation range were evaluated preoperatively and one month after surgery.

**Results:** Mean preoperative OSI was 6.15 ± 2.8 decreasing to 2.3 ± 1.26 one month after surgery. Mean preoperative MTF was 10.03 ± 6.4 cpd increasing to 22.75 ± 10.03 cpd after surgery. Mean preoperative accommodation was 2.4 ± 0.7 D decreasing to 1.3 ± 0.7 D after surgery. These three variables differences were statistically significant (p < 0.001).

Postoperative OSI was significantly correlated with postoperative MTF (r = −0.75) (p < 0.001). Postoperative accommodation range was correlated directly to IOL power (r = 0.42) (p = 0.02) and inversely to axial length (r = −0.39) (p = 0.03).

**Conclusions:** Optical quality improved after surgery. Postoperative accommodation range was correlated to IOL power and axial length.
14
Effects of Posterior Capsule Opacification in Macular Thickness Measurements by Spectral Domain OCT
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Purpose: To study the influence of posterior capsular opacification (PCO) over the measurements of macular thickness performed by means of spectral domain OCT.

Material and Methods: Consecutive patients with previous uneventful surgery cataract, with a monofocal intraocular lens in the capsular bag and with a clinically significant PCO were recruited. Measurements of macular thickness were performed before the capsulotomy by means of spectral domain OCT (Cirrus, software version 6.0, Zeiss). Between 1 and 4 weeks after the PCO removal the same macular OCT measurements were obtained. Comparisons of the corresponding sectors were carried out using the t student test for paired samples.

Results: 35 patients were enrolled in the study. In the comparisons of thickness all the parameters were higher after capsulotomy than they were before (p < 0.05 for all pairs). Considering only patients with signal strength ≥6 there were no significant differences in the measurements before and after PCO removal.

Conclusions: PCO may affect the results of spectral domain OCT. However when considering only reliable tests the differences disappear.

15
Tear Dynamics after Substitution of Antiglaucomatous Therapy with Preservative-free Prostaglandins
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Purpose: To assess whether the substitution of antiglaucomatous therapy with preservative-free prostaglandins drops improves tear dynamics.

Material and Methods: We performed a prospective pilot study on patients treated with latanoprost, travoprost or bimatoprost and dry eye symptoms. Goldmann IOP, Schirmer test, tear clearance and function test (TFI = Schirmer/clearance) and a validated questionnaire for monitoring patients with ocular surface disease symptoms were performed at baseline. Antihypertensive treatment was then changed to preservative-free tafluprost, one drop/24 hours. One month later all test were repeated. Statistical analysis was performed using the Wilcoxon test and Spearman correlation coefficient.

Results: 28 eyes from 14 patients were included. After one month, IOP remained stable. [Baseline IOP = 20.4 ± 2.2 mmHg/1 month IOP = 19.96 ± 2.6 mmHg (p = 0.26)]. Dry eye symptoms improved significantly. [Baseline scores = 9.7 ± 3.7/Rating mon-

th = 5.4 ± 2.7 (p < 0.001)]. No significant differences in clearance (p = 0.1), Schirmer (p = 0.36), TFI (p = 0.06) were found. [Initial clearance = 0.13 ± 0.07/1 month clearance = 0.1 ± 0.07; Initial Schirmer = 10.7 ± 6 mm; 1 month Schirmer = 9.5 ± 3.9 mm. Initial TFI = 80 (48–156). 1 month TFI = 104 (48–216)].

Conclusions: The change to preservative-free tafluprost significantly improved all dry eye symptoms. One of the reasons can be the improvement on tear clearance.

16
Fundus Photography for the Diagnosis of Diabetic Macular Edema
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Purpose: To validate the use of fundus photography for the diagnosis of diabetic macular edema.

Material and Methods: Cross-sectional observational study in 420 eyes of patients with diabetic retinopathy. Different techniques of fundus photography (simple, light and stereoscopic aneritra) and two different fields of view (45 and 30º) were used. Photographs were evaluated by three experts and the results were analyzed with the SPSS 15.0 program. Sensitivity, specificity, positive and negative predictive values and the correlation with the gold standard test (optical coherence tomography) were calculated for each technique.

Results: Sensitivities were below 80% for simple light photographs and above 80% for stereoscopic photographs, while 30º green stereoscopic photographs showed a sensitivity of 94.3%. The specificity was 95% for both color and green stereoscopic photographs of 45º and 30º. The positive predictive value was greater than 95% and the negative value greater than 90% with a coefficient of agreement of 80% and a degree of consistency with the benchmark of over 80%.

Conclusions: The results show that stereoscopic fundus photography can be used reliably for the screening of diabetic macular edema. The use of green filter and its combination with visual acuity can improve the detection of the disease.

17
Dynamic Correlation Between Retinal and Brain Neuroimaging in Multiple Sclerosis
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Castellón General Hospital, Spain

Purpose: To find an exact overlay of the functional activity with the Optical Coherence Tomography retinal images that may reflect the true functional impact of microstructural brain alterations in vivo for better managing of multiple sclerosis patients.

Material and Methods: Retinal structural changes by Optical Coherence Tomography and brain structural changes by Magne-
tic Resonance Imaging were compared in 35 patients at baseline and one-year follow-up to assess clinical syndromes, relapsing-remitting and progressive forms of multiple sclerosis.

Brain cortex volume, retinal nerve fiber layer thickness and macular volumes were recorded. The SIENA automatised program calculated percentages of brain grey substance changes between the two study time points.

**Results:** We found a significant correlation between the relative change in brain grey substance volume and retinal nerve fiber layer from baseline to 1 year follow-up (Pearson R = 0.458, p = 0.019).

**Conclusions:** Integrated medical treatment might include effective ophthalmologist intervention to better manage multiple sclerosis patients. We propose a complete OCT examination for the early detection of brain atrophy in patients with multiple sclerosis at risk for visual impairment related to their neurologic disability. Further research is needed for detecting clinical and preclinical signs and symptoms to help multiple sclerosis patient outcomes.

### 18 Dosing Strategy of Ranibizumab in Retinal Vein Occlusion

**Purpose:** To evaluate the safety/efficacy of intravitreal ranibizumab injection for eyes with macular edema (ME) secondary to retinal vein occlusion (BRVO) and central retinal vein occlusion (CRVO).

**Methods:** All cases with naïve ME secondary to BRVO and CRVO were retrospectively analyzed. An intravitreal injection of ranibizumab 0.5 mg was administered at baseline. Thereafter patients were scheduled monthly and retreated according to a “pro re nata” (PRN) dosing strategy. No other treatment was associated.

**Results:** Forty-five eyes of forty-five patients with BRVO were included. The Snellen mean best-corrected visual acuity (BCVA) improved from 0.08 ± 0.07 to 0.29 ± 0.27 (p = 0.002). Statistically significant improvement in the life quality questionnaires (VF-14).

**Conclusions:** Intravitreal ranibizumab injections are safe and effective for the treatment of ME secondary to BRVO and CRVO in a monthly PRN dosing strategy.

### 19 Intraoperative Ultrasound for Correct Plaque Placement in Choroidal Melanomas

**Purpose:** Plaque brachytherapy placement in choroidal melanomas is usually performed using transillumination. However, this technique cannot determine accurately tumor margins in small size amelanotic choroidal melanomas.

**Material and Methods:** Since 1997 and till 2013, we have used intraoperative transillumination and ultrasound imaging to achieve correct placement of plaque brachytherapy. Using these techniques consecutively, we have treated 101 choroidal melanomas. Around 20% of the tumors were amelanotic, and some were small and located in posterior pole. In thirty-five percent of the cases, the plaques had to be repositioned intraoperatively when transillumination and ultrasound imaging showed different tumor margins.

**Results:** All treated cases showed correct plaque placement after surgery. This ensures the proper tumor treatment and, at a mean follow-up of 33 months, no local tumor recurrence and/or metastatic disease were noted.

**Conclusions:** Intraoperative ultrasound imaging facilitates the identification of choroidal melanoma margins and permits correct placement of the plaque at the time of surgery.

### 20 Lanreotide Autogel for the Treatment of Persistent Diabetic Macular Edema

**Purpose:** To evaluate the efficacy of lanreotide Autogel (long-acting somatostatin analog) in the treatment of persistent diabetic cystoid macular edema (CME).

**Material and Methods:** Thirteen diabetic patients with chronic CME refractory to conventional therapies were treated with monthly 90 mg subcutaneous injections of lanreotide Autogel (Somatuline Autogel, Ipsen Pharma SA, Spain), and were monitored for one year. Outcome measures were best corrected visual acuity (BCVA) measured by a log MAR chart and macular thickness documented with spectral-domain optical coherence tomography (Cirrus HD-OCT). Impact on quality of life was assessed by using two questionnaires on mental health (SF-12v1) and visual function (VF-14).

**Results:** BCVA at 12 months improved in 20 of 26 eyes (76.9%), with a mean increase of 8.62 letters (P = 0.002). Statistically significant improvement in the life quality questionnaires was seen. Retinal thickness changes occurred in 23 of 26 eyes (88.5%) with a mean decrease in macular thickness of 105.96 μm (P = 0.001). Mental health and visual function did not change.
Conclusions: Treatment of persistent CME with lanreotide Auto gel showed statistically significant improvement in both BCVA and macular thickness. Our results suggest that it may be an effective alternative treatment in patients with diabetic CME refractory to conventional therapies.

Purpose: To evaluate the repeatability of the Oculus Pentacam system and to establish the normal aberration values of the anterior corneal surface with the newest software for this system.

Material and Methods: Corneal topography was measured using the new Pentacam Oculus software (version 1.17r139) in 147 normal eyes from 76 patients. The mean age of the patients was 36.49 ± 10.63 years (Mean ± SD), the mean spherical equivalent −2.71 and the mean visual acuity 1.258 ± 0.016. Root mean square values (RMS) and Zernike coefficients were evaluated, focusing specially on RMS Higher-Order Aberrations (RMS HOA), vertical and horizontal coma (Z3 1 and Z3 –1) and spherical aberration (Z4 0).

Results: We describe the aberration values of the anterior corneal surface in normal eyes. Our results differed from those obtained in other studies, due probably to the different software and methods used. The repeatability of the new software was very good (CCI > 0.7) for all the parameters studied.

Conclusions: The newest version of the Oculus Pentacam software permits the exact determination of the normal aberration values of the anterior corneal surface and offers a very good repeatability.

Abstracts
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Ultrastructural Changes and Protection in RPE Cells Cultured in High Levels of Glucose
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**Purpose:** The aim of the present study was to evaluate protective properties of docosahexaenoic acid (DHA) at RPE cells exposed to high glucose levels.

**Material and Methods:** Human RPE cells (ARPE-19) were cultured 4 days with normal blood glucose concentration, followed by ten days exposure to either normal or high D-glucose concentrations. DHA was added giving the following groups: control group (C), control+DHA group (CDHA), glucose group (G) and glucose+DHA group (GDHA). Cells were washed, sonicated and lysed and the homogenates cryopreserved. Cell populations of different groups were evaluated by transmission electron microscopy (TEM).

**Results:** DHA normalized the alterations induced by high D-glucose concentrations. It was detected 0.51 ± 0.07 mmol/mg prot (GSH) in GDHA group. When VEGF and Nrf2 immunohistochemical studies were performed, DHA restores VEGF to control values in GDHA group as compared with G group. Moreover, Nrf2 expression in GDHA group increased significantly when compared to C group showing a possible protection response via Nrf2 pathway. Finally, a TEM study was done to establish possible alterations in the ultrastructure of RPE diabetic cells.

**Conclusions:** These data support previous findings suggesting that DHA might be useful in the protection of the diabetic retina.

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Retinal Degeneration is Accelerated by Low-Dose Ethanol Consumption
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**Purpose:** The aim of this study was to assess whether low doses of ethanol (0.2 to 0.6 g/kg/day) significantly affect retinal degeneration in P23H rats, an animal model of retinitis pigmentosa.

**Material and Methods:** Homozygous P23H (line 3) rats were cultured 4 days with normal blood glucose concentration, followed by ten days exposure to either normal or high D-glucose concentrations. DHA was added giving the following groups: control group (C), control+DHA group (CDHA), glucose group (G) and glucose+DHA group (GDHA). Cells were washed, sonicated and lysed and the homogenates cryopreserved. Cell populations of different groups were evaluated by transmission electron microscopy (TEM).

**Results:** DHA normalized the alterations induced by high D-glucose concentrations. It was detected 0.51 ± 0.07 mmol/mg prot (GSH) in GDHA group. When VEGF and Nrf2 immunohistochemical studies were performed, DHA restores VEGF to control values in GDHA group as compared with G group. Moreover, Nrf2 expression in GDHA group increased significantly when compared to C group showing a possible protection response via Nrf2 pathway. Finally, a TEM study was done to establish possible alterations in the ultrastructure of RPE diabetic cells.

**Conclusions:** These data support previous findings suggesting that DHA might be useful in the protection of the diabetic retina.

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Neurotoxic Effect of Glutamate Receptor Agonists on Inner Retinal Cells
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**Purpose:** Glutamate agonists such as kainic acid (KA) or N-methyl-D-aspartic acid (NMDA) have been used for induction of neuronal toxicity. Our work addresses the neurotoxic effect of KA and NMDA on inner retinal cells.

**Material and Methods:** Retinal cell neurotoxicity was induced in the eyes of C57BL6 mice by intraocular injection of 1 μl of PBS containing increased concentrations of KA and NMDA. Retinal functionality was assessed by electoretinogram recordings (ERG) from control and damaged eyes. Surviving ganglion cell numbers were determined by counting the Brn3a immunolabeled cells. Moreover, specific cell types were detected in retinal sections using immunohistochemistry.

**Results:** Injection of increased concentrations of KA or NMDA induces selective death of retinal ganglion cells. ERG recordings from NMDA or KA injected animals suggested ganglion cell loss. When NMDA and KA were injected together, an abrupt disruption of the b-wave ERG occurred indicating damage of the inner retinal cells. Structural modifications affecting all inner retinal cells were observed by immunolabeling of retinal transverse sections.

**Conclusions:** The present work shows that NMDA and KA glutamate receptor agonists induce a severe damage of the inner retinal cells when injected together into the vitreous.

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Blue Light-Absorbing Filters Does not Affect Circadian Activity in Control and Blind Mice
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**Purpose:** Blue light has been identified as one of the main causes of phototoxicity-induced retinal damage. However, short-wave-length lights are necessary to stimulate melanopsin, which is expressed in the intrinsically photosensitive retinal ganglion cells (ipRGC) to regulate the circadian activity in mammals. In the present work we studied the light entrainment of control and blind mice under the effect of blue filters.

**Conclusions:** Our data demonstrate that low doses of ethanol consumption accelerate retinal degeneration and the loss of retinal function in the P23H rat.

**Support:** MINECO/FU2012-36845, RETICS RD12/0034/0010, ONCE.
Material and Methods: Circadian activity was assessed in mice by wheel-running activity cages. Experiments were performed under white light and under the effect of blue filters of different filtering wavelength. Two different mouse strains were used: C57BL/6J control mice, Rd10 mouse model of rod and cone degeneration.

Results: Both animal strains are able to entrain light induced circadian activity either under control white light or under the effect of blue filtering light. Moreover, ipRGC are sufficient to re-synchronize of endogenous cycles with the circadian activity of each individual even under the effect of blue filters.

Conclusions: When the blue light is not present in the spectral visual sensitivity, the circadian activity is not altered. Different strains of mice are able to synchronize their circadian rhythms without receiving blue light.

p53 Gene Protects the Retina by Regulating Endogenous Oxidative Stress
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Purpose: Age-related macular degeneration (AMD) is a major cause of visual impairment/blindness in older adults. p53 gene encodes for proteins that regulates cell cycle. We analyze the role of p53 gene in relation to redox activity and the molecular events associated with retinal angiogenesis and neuroprotection.

Methods: Groups of adult C57BL/6 mice consisted of transgenic mice with two supernumerary copies of p53 gene (sp53-tg/tg; n = 15) and wild-type mice (wt; n = 15). Retinas were homogenated and used for the determination of lipid peroxidation [malondialdehyde (MDA)] and the total antioxidant activity (TAA). Vascular endothelial growth factor (VEGF) and brain derived neurotrophic factor (BDNF) were determined by ELISA and western blot/immunoblotting. Statistics were performed (SPSS 15.0 software).

Results: A four-fold greater amounts of TAA levels (p < 0.001) and a significant increase in MDA/BDNF (p < 0.01) as well as a significant augmentation of VEGF and BDNF expression were seen in the sp53-tg/tg retinas than in the wt (p < 0.001).

Conclusions: Increased p53 gene expression appears to regulate the sensitivity to endogenous oxidative stress and controls VEGF/BDNF production/availability. We speculate that p53 gene biotherapy may be an alternative approach for potential new diagnostic-therapeutic-prognostic tools for AMD.

Support: RETICS RD 07/0062/0013.

OCT Findings and Autofluorescence in P23H Line 1 Rats
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Purpose: To evaluate FAF and OCT changes in the P23H rat.

Material and Methods: Twenty albino homozygous P23H line 1 rats aging from P18 to 27 months and albino SD rats (2 and 15 months old) were used for this study. SLO imaging, OCT, FAF were acquired using a Spectralis system. A single OCT line was performed passing through the optic nerve of the rat.

Results: During the course of degeneration, the FAF pattern varied from not findings in young animals, some spotting at P60 to a mosaic of hyperfluorescent dots in older rats. Retinal thicknesses diminished during time. Young SD rats showed thickness values higher than old ones (205.2 vs 183.18 μm, at 2 and 15 months old respectively). P23H rats showed great changes in morphology with age. Mean retinal thickness values varied from 189.88 μm to 73.55 μm at 2 months old and 1 months old respectively, lowering till 58.15 μm at 27 months old.

Conclusion: Autofluorescent ophthalmoscopy is a non-invasive procedure that can detect changes in metabolic activity at the RPE in vivo. Hyperautofluorescent changes appeared with the course of the degeneration with a diminution of retinal thickness.

Glucoma Patients and Dry Eye Disorders
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Purpose: Our aim is to understanding Dry Eye Disorders (DEDs) pathogenic mechanisms in relation to primary open-angle glaucoma (POAG). Here we analyze the expression of inflammatory/immune response (IIR) molecules in tears from DEDs and POAG patients.

Material and Methods: Three groups were studied: 1) non-severe DEDs patients (DEDG; n = 30), 2) POAG patients (POAGG; n = 31) and 3) healthy controls (CG; n = 36). An interview and ophthalmic examination were performed. Reflex tears were collected by capillary tubes and processed by a multiplexed particle-based flow cytometry assay for determining a specific set of cytokine/chemokine. SPSS 15.0 program was used for statistical analysis.

Results: Clinical probes manifested significant differences among the DEDG and the POAG (Schirmer test: 4.26 ± 0.59 vs 7.82 ± 1.92 mm) and the controls (13.25 ± 2.46 mm). Differential expression of IIR molecules was detected in tears from the three groups. Both the DEDs and POAG patients manifested increased IL-1β, IL-4, IL-6, IL-8, IL-12, VEGF tear levels vs the CG (p < 0.05).

Conclusions: Chronic instillation of anti-hypertensive eye drops has to be considered for integrating protocols to glaucoma standards of care.
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Metabolomics of the Aqueous Humor in Glaucomatous Rats
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Purpose: Protons available in metabolites can be managed by high resolution nuclear magnetic resonance (1H-NMR) spectroscopy, to simultaneously quantify metabolites within biological fluids, according to their chemical shift. Metabolomic profile of the aqueous humor was assessed in a chronic glaucoma rat model.

Material and Methods: Wistar rats (aged 6 months) were weekly injected in the anterior chamber with either high density hyaluronic acid (left eyes) or balanced salt solution (right eyes). Four more rats were used as the controls. Intraocular pressure and electrorretinograms were performed. After euthanized, rat aqueous humor (AH) was prepared to 1H-NMR spectroscopy, while the retina and optic nerve were processed to morphologic/morphometric/imunohistochemistry, western blot and proximity ligation assays. Statistical analyses (SPSS 15.0) were done.

Results: Microscopic data mimics glaucomatous retinal-optic nerve changes, validating our rat chronic glaucoma model. A database of 1H-NMR from various AH metabolites were obtained. Compounds identified as alanine, valine, lysine, leucine, tyrosine, fenilalanine, N-acetil glutamate, acetocacetate, glutamine, taurine, oxo-glutarate, glucose, VLDL cholesterol, and lactate correlated well with data from conventional techniques, but significantly different relative intensity values were found in the AH spectrums from the glaucoma eyes, as compared to both the sham-operated and control rats.

Conclusions: Chronic glaucoma induces striking changes in the AH metabolic profile.

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Interaction of KCNQ5 and Calmodulin During Retinal Degeneration
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Purpose: To identify the presence of the potassium channel KCNQ5 in the rat retina and evaluate specific interaction between KCNQ5 and calmodulin (CaM) during retinal degeneration.

Material and Methods: Retinas from Sprague-Dawley and P23H line-1 transgenic rats from postnatal day (P) 10 to P160 (provided by Dr. M. La Vail) were processed for KCNQ5 using immunocytochemistry, western blot and proximity ligation assays.

Results: Analysis of retinal sections showed localization of KCNQ5 immunoreactivity in the inner segment of photoreceptors, in the outer and inner plexiform layers and in the ganglion cell layer (GCL). Colocalization and physiological interaction of KCNQ5 with CaM were found in the GCL. Although the KCNQ5 expression decreased in adult P23H rats, the levels of interaction between both proteins, KCNQ5 and CaM, increased with the course of degeneration. These levels were low in young P23H rats, with unappreciated morphological degeneration, and maximal at P160 when the photoreceptors have completely disappeared.

Conclusions: Our results in the rat retina support studies showing that CaM acts as a mediator in modulation of KCNQ5 channels. Moreover, the molecular interaction between KCNQ5 and CaM could be a marker for the progress of retinal degeneration and, somehow, it could affect the cellular maintenance in the inner retina.

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Carbon Nanomaterials for the Reinforcement of the Ocular Tissues
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Purpose: To assess if carbon nanomaterials were safe for the corneal tissue and also to evaluate the mechanical properties of the cornea after the implantation of carbon nanomaterials.

Material and Methods: Experimental protocol where 24 eyes from 12 white rabbits implanted with a composition containing a mixture of Graphene and Carbon Nanotubes after a creation of a pocket in the corneal stroma. Pathology evaluation and mechanical properties of the samples obtained after three months were analyzed in order to determine the foreign body reaction and the modulus of elasticity of the corneal tissue.

Results: Blue Alcian staining showed that there is no fibrous scarring and no alterations in the mucopolysaccharides of the corneal stroma. It also shows that there are no signs of active inflammation after implantation of the carbon nanomaterials. Masson trichrome staining showed that there is no inflammation and no foreign body giant cell reaction. Evaluation of the modulus of elasticity shows a trend towards a higher rigidity in the samples implanted with the carbon nanomaterials.

Conclusions: Carbon nanomaterials (Graphene and Carbon nanotubes) are safe for the corneal tissue and increase the stiffness within the corneal stroma.

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CRB2 and CRB3 Expression in the Adult Mouse RPE
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Purpose: To investigate the putative expression of the members of the cell polarity proteins family CRB in the adult mouse retinal pigment epithelium.

Material and Methods: We have generated and characterized two new antibodies which specifically recognize CRB2 and CRB3 proteins and we have performed RT-PCR, WB and IHQ assays to determine the expression of these proteins in the RPE.
Results: The antibodies designed by our group specifically recognize CRB2 and CRB3 proteins. Both, CRB2 and CRB3 proteins, but not CRB1, are expressed in the adult mouse RPE. CRB2 is located in the RPE tight junctions. In the neural retina, CRB2 is expressed only in Müller cells and it is located in the subapical region of the outer limiting membrane and in the glial element of the synapsis in the outer plexiform layer.

Conclusions: The transmembrane proteins CRB2 and CRB3 are two members of the CRB cell polarity complex expressed in the adult mouse RPE.

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Phagocytic Microglia in Both Eyes after Unilateral Intraorbital Optic Nerve Transection
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Purpose: To analyze the phagocytic microglia after intraorbital optic nerve transection (IONT) in injured and contralateral retinas treated with BDNF or vehicle.

Material and Methods: The left optic nerve (ON) of adult pigmented mice was transected at 0.5 mm from the ON head. Immediately after, the left retinas were intravitreally injected with BDNF or vehicle. One week before surgery retinal ganglion cells (RGCs) were traced by applying OHSt to both superior colliculi. From 3–14 days after IONT, flat-mounted retinas were immunodetected with Iba1 and Brn3a. Iba1+ and OHSt-filled microglial cells (phagocytic) in the ganglion cell layer were semi-automatically quantified and their distribution assessed by neighbor maps.

Results: In the injured retinas, the number of phagocytic microglial cells increased with time after IONT from central to peripheral areas. This response was attenuated by BDNF. In the contralateral retinas, phagocytic microglial cells appeared at 3 days after IONT and their number and distribution was constant up to 14 days after IONT, regardless of the treatment.

Conclusions: After unilateral IONT there is a strong contralateral response that involves microglial activation and OHSt phagocytosis which is not attenuated by neuroprotection of the injured eye and does not concur with quantifiable RGC loss.

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Automatic Analysis of the Rings of Cone Degeneration in the P23H Rat
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Purpose: To analyze the morphological characteristics of the rings devoid of S- and L-cones in the P23H rat retina.

Material and Methods: In whole mounted retinas from homozygous albino female P23H-1 rats, the characteristics of the rings of cone degeneration were analyzed automatically using Image Pro® Plus (IPP). The total number of rings as well as their location, area, perimeter, diameter and epicentre X–Y coordinates (with respect to the optic disk) were determined.

Results: At P180 the normal mosaic of both L- and S-cones in P23H-1 rats is altered by the appearance of ring-like shaped areas devoid of cones. There were 75 ± 46 (mean ± SD) rings per retina and the majority of the rings were located at the equatorial retina. Although the distribution of the rings did not differ between the retinal quadrants, the mean area and diameter of rings were significantly larger in the dorsal than in the ventral region.

Conclusions: The pattern of cone degeneration in the P23H retina suggests differences in the distribution of cone cell death within the retina and deserves further investigation.

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BDNF Delays Ocular Hypertension-Induced RGC Death in Rat
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Purpose: To study the effect of brain-derived neurotrophic factor (BDNF) on the survival of retinal ganglion cells (RGCs) in a rat ocular hypertension (OHT) model.

Material and Methods: The left eyes of albino Sprague-Dawley rats received a single intravitreal injection of 5ug of BDNF or vehicle and laser photocoagulation of the trabecular meshwork, limbar and episcleral veins. Intraocular pressure (IOP) was measured with a TonoLab®. Animals were analyzed at 12 or 15 days after OHT. To assess the impairment of RGC axonal transport caused by OHT, fluorogold was applied to both superior colliculi one week prior to animal processing. All retinas were dissected as flat mounts and Brn3a immunodetected to label surviving RGCs. Total numbers of FG+ and Brn3a+ RGCs were automatically quantified.

Results: In all retinas the percentage of FG+RGCs with competent retrograde axonal transport was approximately 20–25% of the original population. However, the population of Brn3a+RGCs was significantly greater after BDNF treatment than after vehicle treatment both at 12 (82% vs 42%) or 15 (68% vs 49%) days after OHT-induction, respectively.

Conclusions: A single injection of BDNF delays OHT-induced RGC death but does not diminish the axonal damage caused by the elevated IOP.
Conclusions: In the mouse, approximately one third of the cone population are genuine S-cones, one third genuine L-cones and the remaining third dual cones. This cone distribution differs from the rat, in which the dual population is smaller and the S-cones are densest in the retinal periphery.

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Displaced Retinal Ganglion Cells in the Adult Albino and Pigmented Rats
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Purpose: To determine the number and spatial distribution of displaced retinal ganglion cells (dRGCs) in pigmented (FVG) and albino (SD) rats.

Material and Methods: RGCs were labelled with fluorogold (FG) applied to the optic nerve to detect all dRGCs or to both superior colliculi (SCI) to assess how many project to these areas. Retinas were flat-mounted and photographed focusing in the GCL. Each retina encompassed 165 frames which were later reconstructed as a retinal photomontage. By changing the microscope focus from the GCL to the IPL and INL, dRGCs were detected and their position dotted on the photomontage. The number of dots representing dRGCs was automatically quantified and their position determined and illustrated by neighbor maps.

Results: The total number of dRGCs was 395 ± 80 or 2,140 ± 197 in the albino or pigmented strain, respectively. In both strains ~99% of the dRGCs projected to the SCI. dRGCs are observed throughout the retina, though in the dorsal retina they were scarce and their highest concentration was observed in the temporal region.

Conclusions: Almost the whole population of dRGCs projects to the SCI, their numbers are higher in pigmented than in albino rats, and there are mainly located in the peripheral-temporal retina.

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Tauroursodeoxycholic Acid (TUDCA) Prevents Microglia Activation in the P23H Rat Retina
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Purpose: Chronic microglia activation is associated with neurodegenerative diseases and it has been shown that they may contribute to retinal tissue damage. The objective of this study is to quantify microglia activation in the P23H rat retina and to evaluate the neuroprotective effects of TUDCA.

Material and Methods: Homozygous P23H line 3 rats were used. Animals from P20 to P120 were injected weekly with TUD-
CA (500 mg/kg, i.p.). SD rats were used as control. Vertical retinal cryostat sections were immunostained for specific markers of microglia.

**Results:** Microglial cells in the SD rat retinas were distributed in plexus located at the plexiform and ganglion cell layers (GCL). In the P23H retina, an increase in microglia cells numbers was found in all layers compared with control rat retinas. In addition, microglial cells with macrophage-like morphology were observed in the P23H subretinal space, but absence in SD rats. Retina of TUDCA-treated animals exhibits lower cell numbers in all layers and absence of microglia in the subretinal space.

**Conclusions:** Our work suggests that, besides its neuroprotective effect on photoreceptor cells, TUDCA prevents microglial activation in retinitis pigmentosa. Therefore, TUDCA could be potentially useful for future treatments.

**Support:** MINECO BFU2012-36845, RETICS RD12/0034/0010, ONCE.

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**Computer Screens and Dry Eyes**

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**Purpose:** Eye strain, blurred vision and headaches commonly occur in employers using computer screens. We are interested in evaluating the prevalence of dry eye disorders (DEDs) and inflammation biomarkers expression in tears from office workers.

**Material and Methods:** The study involved 800 employers at the Public Administration Offices (Valencia, Spain). Environmental parameters (light/humidity/CO2/air conditioning/dry temperature) were strictly controlled. 81 participants were interviewed and ophthalmologically examined to assess the integrity of the ocular surface, and then distributed into three groups: mild DEDs (DEDG1; n = 58), moderate DEDs (DEDG2; n = 23) and non-exposed employers (CG; n = 35). Schirmer test (Sch), blinking frequency (Bf) and tear collection was done. Tears were subjected to a multiplexed particle-based flow cytometry assay for determining a cytokine/chemokine set. Data were statistically processed (SPSS 15.0 program).

**Results:** The severity of DED prevalence in this employers (mean age 53 ± years) was 72% DEDG1 (Sch : 8.51 ± 4.85; Bf : 9.61 ± 4.98) and 28% DEDG2 (Sch: 4.20 ± 3.36; Bf: 8.74 ± 4.07 mm). Significant different expression of GM-CSF, TNFa, VEGF, IFg, IL1b, IL-2, IL-6, IL-8, and IL-10 in the DEDG1/DEDG2 vs the CG (P < 0.001) was also observed.

**Conclusions:** The prevalence of DEDs complains will lower with a computer-vision-benefit-program.

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**Non-ocular Injuries Can Activate Mouse Retinal Microglia**

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**Purpose:** To determine whether injuries initiated in non-ocular tissues (such as systemic fungal infection, immunosuppression and/or peripheral inflammation) may cause activation of retinal microglia.

**Material and Methods:** Invasive candidiasis, colitis induced by dextran sulphate sodium salt, and immunosuppression by cyclophosphamide treatment were performed in murine models. Activation of retinal microglia were analyzed by confocal immunofluorescence analysis to determine microglial cells morphology and immunoreactivity against anti-IBA1 and anti-MHCIIRT1B antibodies.

**Results:** Systemic fungal infection, immunosuppression and, to a lesser extent, peripheral inflammation, caused activation of retinal microglia.

**Conclusions:** Injuries initiated in non-ocular tissues, may represent a risk factor for patients with ocular neurodegenerative diseases, as diabetic retinopathy or glaucoma, due to their potential to activate retinal microglia.

**Support:** MINECO BFU2012-36845, RETICS RD12/0034/0010, ISCIII PI080556 to NC; JCI-2009-05224 to VGV.

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**A New Automatic Method for Counting Microglial Cells in Wholemount Mice Retinas**

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**Purpose:** Microglial proliferation is accepted as a hallmark of glial activation. This study aimed to develop an automatic method that allows assessing the number of retinal microglia in a laser-induced ocular hypertension model (OHT).

**Material and Methods:** Adult Swiss mice were divided in two groups: naive (n = 6) and lasered (n = 6). Retinal whole-mounts were immunostained with anti-Iba-1. A new algorithm was developed in Matlab to obtain the number of Iba-1 microglial cells.

**Results:** In both naive and OHT-retinas the automatic method detected the number of Iba-1+ cells in the inner and outer plexiform layers of the retina. The time required for counting Iba-1+ cells decreased from the human guide to the automatic counting method. There is a strong correlation between both methods indicating the consistency of the automatic counting (Pearson correlation test, R = 0.979; P < 0.0001 and R = 0.942; P < 0.0001 for outer and inner plexiform layer respectively).

**Support:** MINECO BFU2012-36845, RETICS RD12/0034/0010, ONCE.
Conclusions: A new and reliable algorithm has been developed with Matlab to obtain the number of Iba 1+ cells in mice retinal wholemounts. A bigger set of images could be included in future studies to analyze the proliferative behavior of microglial cells.

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The Synthetic Cannabinoid HU210 Slows Retinal Degeneration in P23H Rats
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Purpose: To assess the therapeutic potential of the cannabinoid agonist HU210 in the P23H rat model of retinitis pigmentosa.

Material and Methods: Homozygous male P23H (line 3) rats received HU210 (100 mg/kg, i.p.) or vehicle three times a week from P24 to P90. In these animals, visual function was evaluated by electroretinographic (ERG) recording at P30, P60 and P90. Preservation of retinal structure has been assessed by immunohistochemistry at P90.

Results: In HU210-treated animals, ERG recordings showed an amelioration of vision loss, as compared to vehicle-administered animals. The scotopic b-waves were significantly higher in treated animals than in control rats at P30, P60 and P90 (p < 0.05, ANOVA). This attenuation of visual deterioration correlated with an amelioration of vision loss.

Conclusions: The cannabinoid agonist HU210 preserves retinal structure and function in the P23H rat. This work suggests that cannabinoids are potentially useful to delay retinal degeneration associated with an impairment of ERG and Bcl-2 overexpression. Therefore, these alterations proposed the existence of a potential “alcoholic retinopathy”. Hence, we tested the effect of alcohol exposure to a specific retinal cell type, a human Retinal Pigmented Epithelium cell line (ARPE-19).

Results: Cells were plated at confluence and then exposed to 3 different alcohol concentrations (250 mM, 500 mM and 750 mM). Using MTT and Alamar blue assays, we found out that cell viability was affected by the 3 different concentrations. We checked these results either by flow citometry and fluorescent microscopy. Furthermore, different cell death markers were overexpressed, as much as oxidative stress markers.

Conclusion: Our results indicate that alcohol is metabolized by ARPE-19 cells, leading to an increase of oxidative stress markers and an impairment of cell viability.

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Role of Autophagy During the Development of the Retina
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Purpose: Autophagy is an evolutionarily conserved process that occurs at basal conditions and promotes cellular homeostasis through the recycling of cellular components and supplying energy during periods of metabolic stress. In this work we have studied the role of autophagy in the development of the central nervous system.

Material and Methods: We used as a model the mouse retina in conditions where autophagy is blocked, both pharmacologically (with 3-MA and wortmannin) and genetically using the retina of mice deficient in autophagy Ambra1. Autophagy, cell death and phagocytosis were determined by histologic and biochemical tools.

Results: Pharmacological inhibition of autophagy with 3-MA or wortmannin in mouse retinas in vitro, induces an accumulation of apoptotic cells due to a decreased exposure of phosphatidylserine. Methylpiruvate supplementation restores the presentation of phosphatidylserine on the cell surface. This results in the reestablishment of the degradation of apoptotic cells. In addition we show that retinas from autophagy-deficient mice show several malformations and increased levels of cell death.

Conclusions: Autophagy is essential to provide the energy for cell corpse removal during cell death associated to normal development and has a cytoprotective role during embryonic development.

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Ethanol Exposure Induces Cell Death by Oxidative Stress in a Human RPE Model
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Objectives: Ethanol exerts its deleterious effects metabolically via oxidative and non-oxidative pathways, involving free radical production and lipid peroxidation, potentially leading to an imbalance between oxidants and antioxidants, resulting in an increased oxidative stress.

Methods: Our group demonstrated that chronic ethanol consumption induces toxicity by increasing oxidative stress in rat retina associated with an impairment of ERG and Bcl-2 overexpression. Therefore, these alterations proposed the existence of a potential “alcoholic retinopathy”. Hence, we tested the effect of alcohol exposure to a specific retinal cell type, a human Retinal Pigmented Epithelium cell line (ARPE-19).

Results: Cells were plated at confluence and then exposed to 3 different alcohol concentrations (250 mM, 500 mM and 750 mM). Using MTT and Alamar blue assays, we found out that cell viability was affected by the 3 different concentrations. We checked these results either by flow citometry and fluorescent microscopy. Furthermore, different cell death markers were overexpressed, as much as oxidative stress markers.

Conclusion: Our results indicate that alcohol is metabolized by ARPE-19 cells, leading to an increase of oxidative stress markers and an impairment of cell viability.

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Strategies to Optimize the Sample Preparation for Proteomic Analysis of Vitreous Humor in Retinal Pathologies
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Introduction: The analysis of proteins in human vitreous humor (VH) can elucidate the pathogenesis of retinal ocular diseases and provide information for the development of potential bio-
markers or therapeutic targets. Nowadays, proteomic science has emerged as an analysis technique, offering a suitable mean for study protein levels at normal and disease states. The complexity of this procedure is increased by the high degree of heterogeneity and difficulty of breaking structural interactions between proteins.

**Objective:** Optimization of samples preparation for proteomic analysis of VH in retinal pathologies using two-dimensional electrophoresis and mass spectrometry (MALDI-TOF/TOF).

**Materials and Methods:** Samples of VH from patients with different retinal diseases were used. Several approaches for VH samples treatment such as organic precipitation, pre-fractionation, abundant proteins depletion, ultrasonic and freeze-thaw homogenization were studied. Additionally, stabilization studies of VH samples were performed with buffer containing Tris, denaturing agents (Urea), reducing agents (dithiothreitol) and proteases inhibitors (leupeptin and pepstatin).

**Results and Conclusions:** The use of stabilizers improves the protein solubility and the resolution of the two-dimensional electrophoresis. It can be concluded that an adequate sample preparation is critical to preserve quality and to further successful protein processing and analysis.

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**A New Murine Retinal Cell Line for the Study of Photoreceptor Degenerative Mechanisms**

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**Purpose:** To obtain and characterize a novel retinal progenitor cell line which could serve as an in vitro model for the study of photoreceptor degenerative mechanisms.

**Material and Methods:** A Müller-derived cell line (MU-PH1) was isolated from adult C57BL6 mouse retina. RT-PCR, immunoblot and immunofluorescence analyses were performed to characterize these cells. Light responsiveness was assessed by calcium imaging.

**Results:** Spontaneously immortalized MU-PH1 cell line exhibited glial-like morphology and characteristics of neural retinal progenitors, as identified by their ability to form neurospheres and the expression of neural stem cell markers (nestin, Abcg2, α-tubulin, β-III-tubulin and Ascl1). Additionally, MU-PH1 cells expressed markers of Müller glia (vimentin, S-100, glutamine synthetase) and of differentiated retinal neurons (rhodopsin, recoverin, γ-transducin, melanopsin and cone opsins). Other markers such as CRALBP, GFAP, CD31 or CD11b were undetectable. Stimulation with 480 nm light evoked slow and fast transient calcium responses.

**Conclusions:** MU-PH1 cell line is a new Müller-derived cell line that stably expresses photoreceptor markers as well as glial and stem cell markers. Availability of MU-PH1 cell line may provide a unique tool to study photoreceptor signalling pathways and the efficacy/toxicity of neuroprotective compounds.

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**Autophagy Dysregulation in the Retina: Neurodegeneration and Aging**

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**Purpose:** Accumulation of undegraded proteinaceous material represents a hallmark in many retinopathies, such as age-related ones. Autophagy contributes to maintain cellular homeostasis removing damaged cellular components in lysosomes. Although autophagy alterations underlie the basis of a growing number of human diseases, very little is known about the contribution of this process to the pathophysiology of the retina.

**Material and Methods:** 3–12 and 22-month-old C57B6/J mice and Atg5flx/flx; nestin-Cre 7-week-old mice were used in this study. Autophagy was documented by biochemical, histological and molecular methods tool. Retina degeneration was assessed by histology and electroretinogram.

**Results:** In aged retinas, autophagic flux is less efficient in basal/starvation conditions, associated to increased lipofuscin, ubiquitinated proteins and p62 in all retinal layers. Decreased mRNA expression of the autophagy regulators Beclin1 and Atg7 was also observed. We then analyzed the consequences of macroautophagic blockage in retina with the Atg5flx/flx; nestin-Cre mice model. We observed apoptotic nuclei in the photoreceptor layer and a clear reduction of the visual function at 7 weeks of age, statistically significant in the scotopic tests, in parallel with aging processes.

**Conclusions:** We have identified a primary malfunction of macroautophagy in the aging retina that contributes to the age-associated reduction of visual function.
apoptotic molecule, and 4) the inflammation mediator interleukin-6 (IL-6). Data were statistically analysed (SPSS 15.0 program).

**Results:** The MDA/TBARS positively correlated with increasing PARP-1 and IL-6 and the visual field progression in POAG patients, whereas the TAA negative correlated with these molecules and also with glaucoma clinical parameters of severity. No relationship was obtained from the comparatives.

**Conclusions:** Oxidative stress biomarkers, as well as those related to inflammation and apoptosis may help managing POAG progression. New therapeutic strategies can be developed from our results.

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**Pro-Inflammatory Capacity of ARPE19 Cells in Response to Toll Like Receptors Challenge**

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**Purpose:** Discrimination of potential pathogens with the toll-like receptors (TLRs) is main objective of the innate immune system. This recognition leads to expression of genes such as inflammatory cytokines and co-stimulatory molecules. We analyze the pro-inflammatory (PI) capacity of cultured human retinal pigment epithelium (RPE) in response to TLRs challenge.

**Material and Methods:** A human RPE cell line (ARPE19) was established by trypsinization of a primary RPE culture resulting in a uniform cell population (displaying strong growth potential). We assessed pro-inflammatory (PI) TLRs-induced activation in these cells, by western-blot/immunoblotting, confocal microscopy and flow cytometry of conditioned media, as an immune aggression “in vitro” model. Data were statistically processed (SPSS 15.0 program).

**Results:** Full expression of IL-4 and IL-13 receptors was observed in ARPE19 together with hemeoxygenase-1/arginase-1 formation, these latter being involved in cytoprotection against PI mediators. All data suggest that TLR activation and transcriptional PI cytokines expression-secretion were induced in RPE cultures.

**Conclusions:** Unraveling the regulated activities of TLRs in RPE cells and its plasticity in immune response, new therapeutic avenues to control retinal inflammation may be revealed.

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**Nutraceutics Benefits Dry Eyes by Lowering Inflammation Biomarkers**

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**Purpose:** Inflammation is involved in dry eyes (DE). We evaluate the effects of a combined formulation of antioxidants (AOX) and essential polyunsaturated fatty acids (EPUFAs) in DE.

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**Analysis of USH1 Transcripts from Nasal Epithelial Cells**


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**Purpose:** Usher syndrome type I (USH1) is an autosomal recessive disorder characterized by congenital profound deafness, retinitis pigmentosa and vestibular areflexia. The genes responsible for the disease present a restricted expression profile (photoreceptors and inner hair cells) and human specific tissue samples are difficult to obtain. The presence of eight Usher proteins and the effect of splicing mutations could be observed in nasal epithelial cells.

The aims of this study were to analyze the effect of putative splice-site variants in USH1 transcripts, obtained from nasal epithelial cells of our patients, and corroborate their splicing effect previously observed by minigenes.

**Material and Methods:** Nasal epithelial cells were obtained from five USH1, two family healthy carriers and controls. Analysis of the RNA from nasal cells was carried out in order to observe the effect of mutations on the splicing process.

**Results:** Only in four of the eight studied variants: one MYO7A (c.5856G>A) and three CDH23 (c.2289+1G>A, c.6049G>A and c.8722+1delG) we could observe an abnormal splicing process. The same results were obtained by minigene constructs.

**Conclusions:** The analysis of nasal epithelial cells is an alternative method to discriminate neutral Usher variants from those with a pathogenic effect on the splicing process.
Presence of Monocyte Cells as a Bilateral Response to Unilateral Optic Nerve Lesion
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Purpose: To characterize and quantify the recruitment and trafficking of phagocytic immune cells, circulating between the bloodstream and the neural parenchyma of the contralateral retina to the lesion, in response to unilateral optic nerve crush (ONC).

Material and Methods: Unilateral lesion by micro-ONC to the left optic nerve (ON) was performed on female Spague-Dawley rats. Intracytoplasmic horseradish peroxidase (HRP) was intravenously injected to detect monocyte/macrophage cells trafficking from bloodstream. Based on morphology, Iba1-positive immunoreactive monocyte/macrophages were quantified in whole-mount retinas.

Results: Monocyte/macrophage cells quantification showed two peaks, at 1 or 5 days post-lesion, in the injured and uninjured retinas. Histoenzymatic HRP detection in the monocyte/macrophage cytoplasts in both rat retinas demonstrated the selective permeability of the blood-retinal barrier to permit the passage of this cell type in response to unilateral lesion of the optic nerve.

Conclusions: Unilateral optic nerve micro-crush causes extravasation of monocyte/macrophage cells from the systemic blood circulation, in both the injured and uninjured retinas. The described cellular migration has not previously been published in the contralateral retina to the lesion site.

Preclinical Safety of Intravitreal Docosahexaenoic Acid in a Rabbit Model
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Purpose: To evaluate retinal toxicity of intravitreal docosahexaenoic acid (DHA) in rabbit eyes over a short-term period.

Material and Methods: Sixteen New Zealand albino rabbits were selected. Six concentrations of DHA were injected intravitreally in the right eye of the rabbits: 10 mg/50 μl, 5 mg/50 μl, 2.5 mg/50 μl, 50 μg/50 μl, 25 μg/50 μl, and 5 μg/50 μl. Vehicle solution was injected in one eye of four animals as controls. Retinal toxicology was studied by slit-lamp examination/electroretinography. Rabbits were euthanized after one week follow-up and the eyeballs were processed for histological examination. Aqueous and vitreous humor samples were taken to quantify the concentration of DHA. Retinal toxicology was studied by slit-lamp examination/electroretinography. Monocyte/macrophage cells quantification showed two peaks, at 1 or 5 days post-lesion, in the injured and uninjured retinas. Histoenzymatic HRP detection in the monocyte/macrophage cells trafficking from bloodstream and the neural parenchyma of the contralateral retina to the lesion, in response to unilateral optic nerve crush (ONC).

Results: Our results indicate that intravitreal DHA is safe in the albino rabbit up to the maximum tolerated dose of 25 μg/50 μl. Further studies should be performed in order to evaluate the effect of intravitreal injection of DHA as a treatment of different retinal diseases.
Four weeks after surgery, surviving Brn3a+RGCs and ipRGCs applied to the distal end of the PNG to identify regenerating RGCs.

Results: Approximately 13% of the original Brn3a+RGC population survived after simple ONT, 18% after PNG+suture (p = 0.003 vs ONT) and 22% after PNG+Tissucol® (p = 0.023 vs ONT). The proportion of OHSt+RGCs was higher in the PNG+suture group when compared to the PNG+Tissucol® group (p = 0.002).

Conclusions: In mice, attaching a PNG to the ON stump either by suture or Tissucol® results in significantly greater RGC survival, and promotes axonal regeneration. The latter is higher when the PNG is sutured to the severed optic nerve than when is apposed with Tissucol®.

RGC Regeneration Along Peripheral Nerve Grafts in Albino and Pigmented Rats


Purpose: To study the effect of a peripheral nerve graft (PNG) on the survival and regeneration of axotomized retinal ganglion cells (RGCs) and intrinsically photosensitive RGCs (ipRGCs) in pigmented and albino rats.

Material and Methods: The left optic nerve (ON) of 30 rats (18 albino, 12 pigmented) was intraorbitally transected and divided into two groups: simple axotomy or axotomy plus PNG to the ON stump. Two days prior to sacrifice, fluorogold (FG) was applied to the distal end of the PNG to identify regenerating RGCs. Four weeks after surgery, surviving Brn3a+RGCs and ipRGCs were detected in flat-mounted retinas. All RGCs were automatically quantified.

Results: After axotomy, 2.8% (albino) or 1.4% (pigmented) RGCs survived. After PNG there was a significant increase in the number of Brn3a+RGCs, which was even higher in albino (10%) than in pigmented (6.5%) rats. Likewise, the number of regenerating RGCs was greater in albino (7.042 ± 2.915) than in pigmented (3.724 ± 1.153) rats. However, the number of surviving ipRGCs was similar in both strains and there were no FG-labeled ipRGCs.

Conclusions: The effect of a PNG on the survival and regeneration of RGCs is different in albino and pigmented rats, and while RGCs regenerate through it, ipRGCs do not.

Mouse Retinal Ganglion Cell Loss after Optic Nerve Crush and Transection: A Comparative Study


Purpose: To analyze and compare the temporal course of mouse retinal ganglion cell (RGC) loss after intraorbital optic nerve crush (IONC) or transection (IONT).

Material and Methods: The left optic nerve (ON) of albino Swiss mice was intraorbitally crushed or transected at 0.5 mm from the ON head. At increasing survival intervals (from 3 to 90 days post-lesion, dpl) retinas were dissected as whole flat mounts and subjected to Brn3a immunodetection to identify surviving RGCs. The total number of Brn3a+RGCs was automatically quantified and their distribution assessed by isodensity maps. The right retinas were used as controls.

Results: After both injuries, RGC death was first significant at 3 dpl when approximately 10% of the original RGC population was lost. At 14 dpl, 23% or 21% of them survived after IONC or IONT, respectively. By day 90, only 5% of the RGCs survived after both types of axotomy. Isodensity maps showed that RGC loss is diffuse throughout the whole retina.

Conclusions: In mice, RGC death induced by intraorbital nerve crush or transection causes the same amount of death, as opposed to rat where IONC causes a slower RGC loss than IONT.

Computerized Measurement of Rat Retinal Ganglion Cell Somatic Area


Purpose: To measure and compare the somatic area of retinal ganglion cells (RGCs) in albino and pigmented rats.

Material and Methods: RGCs from 4 albino (SD) and 4 pigmented (PVG) rats were retrogradely labelled with fluorogold (FG) applied to both superior colliculi. Seven days later, retinas were dissected as flat-mounts and photographed. Each retina encompassed 165 frames which were later reconstructed as a retinal photomontage. To identify and measure somatic area of FG+RGCs, new automated image analysis software was developed and validated; briefly after cell identification, soma contour was selected and its inner area measured. Quantitative and qualitative results were exported to a data sheet for further analyses.

Results: In total 266,286 RGCs from albino and 266,526 from pigmented rats were evaluated. In both strains RGC somas ranged from 60–120 μm²; 17.3% (PVG) and 14.2% (SD) between 120–180 μm².

Conclusions: In mice, attaching a PNG to the ON stump either by suture or Tissucol® results in significantly greater RGC survival, and promotes axonal regeneration. The latter is higher when the PNG is sutured to the severed optic nerve than when is apposed with Tissucol®.
and the remaining 10.5% (PVG) and 6.2% (SD) had somas larger than 180 μm².

**Conclusions:** Automated routines are reliable tools to measure and classify rat RGCs by their somatic area.

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**Effect of Selected Polymorphisms on the Risk of POAG**


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**Purpose:** to study the relationship between selected polymorphisms and plasma levels of vitamins, and the effect of these polymorphisms on primary open-angle glaucoma (POAG) risk.

**Material and Methods:** case-control study involving 500 subjects, matched by age and gender. Four polymorphisms were analyzed (SLC23A1 rs10063949, SLC23A2 rs12796833, TTPA rs6994076, SEC14L2 rs737723). Samples were genotyped by means the Taqman allelic discrimination technique. Plasma levels of vitamins were determined by HPLC.

**Results:** the concentration of vitamins in plasma was significantly lower in the glaucoma group than in the control group (vit. C: 10.0 ± 1.6 μg/mL vs. 12.0 ± 1.7 μg/mL respectively, p < 0.001; vit. E: 10.7 ± 1.7 μg/mL vs. 11.4 ± 1.8 μg/mL, p < 0.001). The SLC23A2 and SEC14L2 polymorphisms were strongly associated to high risk of POAG. The G/G genotype of SLC23A2 gene was associated to lower levels of vitamin C. The A/A genotype of TTPA gene was associated to lower plasma levels of vitamin E. The existence of these both risk genotypes increases the risk of POAG.

**Conclusions:** we have found important effects of several genetic polymorphisms on the risk of POAG. These effects may be mediated by the contribution of these SNPs to plasma concentrations of vitamins C and E.

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**Activation of Steroid Receptors Using a Novel Liposomal Antinflammatory Formulation**


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**Purpose:** New liposome-based formulations, used as unprepared artificial tears, may be used as anti-inflammatory drug delivery systems. We aimed to determine the activation of steroid receptors after the treatment with medroxyprogesterone-loaded liposomes (mdx-lipo).

**Material and Methods:** The Human Corneal Epithelial (HCE) cell line was cultured and treated with mdx-lipo for 1h. Then, the protein was obtained from cell lysates. Expression of glucocorticoid and progesterone receptors was determined by Western blotting and immunofluorescent detection using specific antibodies (Ab). Blank liposomes without medroxyprogesterone were used as controls.

**Results:** Western blot analysis revealed that the expression of both receptors was increased after mdx-lipo treatment. In addition, receptors changed their cellular location, as determined by detection of their Ab-associated immunofluorescent signal. Receptor translocation from the cytoplasm to the nucleus was observed indicating activation. However, the activation of glucocorticoid receptor was increased in a greater extent than that of the progesterone receptor.

**Conclusions:** Exposure of HCE cells to mdx-lipo leads to an activation of glucocorticoid and progesterone receptors. Experiments to determine the antiinflammatory activity of mdx-lipo are ongoing using an *in vitro* model of ocular surface inflammation.

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**Evidence for CYP1B1/FOXC1 Digenic Inheritance in a Patient with Axenfeld-Rieger Syndrome and Congenital Glaucoma**

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**Purpose:** To determine the genetic alteration in a patient affected by Axenfeld-Rieger syndrome and primary congenital glaucoma.

**Material and Methods:** We analyzed FOXC1 and CYP1B1 DNA sequences in a patient diagnosed at birth with bilateral primary congenital glaucoma and Axenfeld-Rieger syndrome. Her parents were also genotyped for the identified variants. In order to assess the pathogenicity of the identified FOXC1 polymorphism, it was cloned and both the transcriptional activity and protein stability were studied in vitro.

**Results:** Analysis of both FOXC1 and CYP1B1 genes revealed that the patient carried a CYP1B1 null mutation along with a FOXC1 polymorphism, both in the homozygous state. The progenitors were first cousins and carried each variant in heterozygous state. The functional study showed that the FOXC1 polymorphism moderately increased the transcriptional activity due to increased protein stability.

**Conclusions:** These results provide strong evidence for the existence of digenic CYP1B1/FOX1 inheritance in Axenfeld-Rieger syndrome with primary congenital glaucoma, suggesting the role of the FOXC1 polymorphism as a modifier factor in the proper genetic background.
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Identifying Genes Involved in Primary Congenital Glaucoma Using Whole Exome Sequencing
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Purpose: To identify genes involved in Primary Congenital Glaucoma using whole exome sequencing.

Material and Methods: We selected 21 patients with neonatal bilateral glaucoma diagnosed in the four first months of life with no known CYP1B1 mutations and no family history of glaucoma. Genomic libraries were prepared with the Paired-End Sequencing Library Prep kit. Exome capture was performed with the SureSelect Human All Exon 50 Mb Kit. Sequencing was performed on HiSeq2000 (Illumina). Sequences were mapped and a bioinformatic analysis was performed to identify pathogenic variants. Data were filtered according to the following criteria: coding variants with high functional impact prediction not present in data bases and, recessive inheritance (homozygous or compound heterozygous states).

Results: We detected an average of 59.775 variants per patient. 10.4% of them are unknown variants and only 0.89% were predicted to induce a high functional impact. We identified potential pathogenic variants in 14 genes of 12 patients. Almost all these genes are involved in the embryonic development or in cellular proliferation control.

Conclusions: These preliminary data indicate the existence of high genetic heterogeneity in PCG, although the pathogenicity of the identified variants must be confirmed by both segregation and functional analyses.

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Bicarbonate Regulates Secretion and Proteolytic Processing of the Glaucoma-Related Protein Myocilin
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Purpose: To identify possible factors regulating the proteolytic processing of recombinant myocilin, an extracellular glycoprotein of poorly understood function involved in glaucoma. We have previously shown that recombinant myocilin undergoes an intracellular proteolytic processing by calpain II which cleaves the central region of the protein, releasing one N- and one C-terminal fragment. Myocilin cleavage is reduced by glucocorticoid-associated mutations and it has been proposed to participate in IOP modulation.

Material and Methods: We used HEK 293T and hCM cells as a model to analyze how different culture medium parameters (i.e., culture time, cell density, pH, bicarbonate concentration, etc.) affect the secretion and proteolytic processing of myocilin.

Results: Extracellular bicarbonate depletion associated with culture medium acidification produced a reversible intracellular accumulation of full-length recombinant myocilin and increased its intracellular proteolytic processing. It was also determined that myocilin intracellular accumulation depends on its N-terminal region.

Conclusions: Myocilin secretion is bicarbonate-dependent in this cellular model. Its proteolytic processing is inversely proportional to bicarbonate concentration. These data suggest that sodium bicarbonate variations in the aqueous humour could also modulate the secretion and cleavage of myocilin present in ocular tissues.

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Biodegradable Ketorolac-Loaded Microspheres as Controlled Delivery Systems for Intraocular Administration
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Purpose: Intraocular inflammatory disorders embrace a broad number of diseases. For a successful treatment, frequent intraocular injections of anti-inflammatory drugs are required. Ketorolac is a nonsteroidal anti-inflammatory drug with fewer side effects than corticosteroids. The aim of the present work was to evaluate ketorolac-loaded microspheres of poly-(D,L-lactide-co-glycolide) as controlled delivery systems for long-term therapies.

Material and Methods: Ketorolac-loaded microspheres (K-Ms) were prepared based on the emulsion-solvent evaporation technique. Different ratios of drug to polymer were employed: 0.5:10 (K-Ms1), 1:10 (K-Ms2) and 1.5:10 (K-Ms3). Ms were characterized by particle size, morphology, encapsulation efficiency and in vitro release of ketorolac.

Results: K-Ms were spherical with smooth surface. The encapsulation efficiency higher than 50%: 53.05 ± 2.06% (25.20 ± 0.9 μg K/mg Ms, K-Ms1), 54.58 ± 3.05% (49.32 ± 3.2 μg K/mg, K-Ms2) and 56 ± 2.14% (73.12 ± 2.8 μg K/mg, K-Ms3). Ketorolac release profiles depended on the initial drug:polymer ratios. The initial burst (first 24 h) was 1.05 ± 0.7 μg K/mg Ms for K-Ms1, 3.67 ± 2.9 μg K/mg Ms for K-Ms2 and 8.46 ± 6.6 μg K/mg Ms for K-Ms3. A progressive sustained release was observed during at least 2 months in all cases.

Conclusions: Biodegradable ketorolac-loaded PLGA microspheres are potentially useful as controlled delivery systems for the treatment of inflammation in intraocular diseases.
Outer Retinal Degeneration in a DBA/2J Mice, A Model of Ocular Hypertension

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Purpose: The DBA/2J mouse is a model of ocular hypertension and retinal ganglion cell degeneration. The aim of this study was to evaluate changes in the outer retina of DBA/2J.

Material and Methods: DBA/2J were studied at 3, 8 and 16 months, and C57BL/6 were used as age-matched controls. Retinal cryostat sections were immunostained for specific markers of different retinal cells.

Results: At 3 months, the number of photoreceptor rows in the ONL of DBA/2J and C57BL/6 did not differ. However, pre- and postsynaptic immunostaining showed that horizontal and ON-bipolar cells had less and shorter dendritic processes in the DBA/2J retinas. At 8 months a reduction of the number of photoreceptor rows was detected, being higher at 16 months old. Outgrowth of postsynaptic cell processes was observed at this age accompanied by loss of synaptic connectivity. In DBA/2J, ganglion cells degenerate in patches beginning at 8 months, which are associated with greater retinal degeneration.

Conclusions: In DBA/2J, ganglion cell degeneration is accompanied by photoreceptor death and loss of their synaptic contacts. These changes are exacerbated inside the patches with an accelerated degeneration.

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Physiopathological Retinal Aging

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Purpose: The retina, as the rest of the central nervous system, is subjected to normal, as well as to pathological aging processes. Among others, age-related macular degeneration is a highly prevalent complication of aging and it has been recently connected to Alzheimer disease. We look for common molecular and cellular mechanisms underlying physiopathological aging in the retina and the CNS, as well as for potential therapies to delay the process.

Material and Methods: Immunohistochemistry and electrophysiology were used to evaluate the effects of normal aging in C57BL/6 mouse retinas. As model for pathological aging, SAMP8 mouse retinas were also studied. To assess the effects of a proinsulin treatment in retinal degeneration, we employed intramuscular injection of adenoasociated vectors (AAV) expressing human proinsulin.

Results: Common signs of physiopathological aging were impairment of visual function and reactive gliosis, both partially prevented with human proinsulin expression. Further cellular molecular characteristics are under study.

Conclusions: The description of common mechanisms may assist in the development of novel therapeutical approaches to retinal dystrophies.

Clinical Cases

Polypoidal Choroidal Vasculopathy as a Complication of Dome-Shaped Macula

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Introduction: To describe polypoidal choroidal vasculopathy (PCV) as a complication of dome-shaped macula.

Clinical Case: A 54-year-old woman presented with metamorphopsia and visual loss in the right eye (OD). Best-corrected-visual-acuity (BCVA) was 0.2 OD and 1.0 OS. Complete ophthalmic examination that included fluorescein angiography, spectral-domain optical coherence tomography (SD-OCT), fundus autofluorescence and indocyanine green angiography (ICG-A) was performed. SD-OCT showed changes at the level of retinal pigment epithelium, marked cystoid edema and images compatible with polyps. The ICG-A study confirmed the presence of polyps on the edge of a choroidal vascular network located at the center of the macula on the top of the dome.

The patient was treated for three years with repeated ranibizumab injections and twice with photodynamic therapy, with a chronic and recurrent evolution. After the last treatment the patient maintained a BCVA of 0.3 with normalization of tomographic images.

Conclusion: Choroidal abnormalities at the center of the dome-shaped macula may induce the appearance of PVC. Response to treatment can be difficult as we have observed in our case.

SD-OCT in Diagnosis and Follow-Up of Optic Nerve Head Drusen (ONHD)

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Introduction: ONHD occur in 3.4–24 per 1,000 population and is bilateral in approximately 75%. It consists of an accumulation of calcium resulting from axonal degeneration. Unburied...
ONHD are very rare in children. ONHD change their appearance with age, increasing their size and visibility. In adults, ONHD cause nerve-fiber-bundle defects in automated perimetry (AP). In some cases, it can be mistaken for papilledema. ONHD are autofluorescent and appear as well-defined uneven hyperfluorescence in fluorescein angiography. The diagnosis of ONHD is confirmed by B-scan ultrasound. SD-OCT provides high-resolution images of the optic nerve head.

**Case Presentation:** A 30-year-old male underwent a routine examination. His best-corrected visual acuity was 1.0 OU. Slit lamp and AP scores were within normal limits. Fundoscopy examination revealed superficial ONHD OU, which were autofluorescent. On SD-OCT, ONHD appeared as globular bodies protruding from the disc, giving rise to an irregular, indistinct disc margin and high reflectivity casting a shadow underneath that obscured the RPE band.

**Conclusions:** B-scan echography has been considered the most reliable method in drusen detection. However, OCT has proven to be a very sensitive and specific diagnostic method and may become a reliable method in drusen detection. However, OCT has proven to be a very sensitive and specific diagnostic method and may become a useful tool in documenting the changes seen in retinal fiber layer.

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**Fundus Albipunctatus and Cone Dystrophy**

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**Introduction:** Fundus Albipunctatus is a form of stationary night blindness with autosomal recessive inheritance, where the nyctalopia is adaptative, without macular impairment.

**Case Presentation:** A 42 years old female, healthy without family history of night blindness . She complained of stationary night blindness which reversed after adaptation to darkness. No low vision or scotomas were referred, The BCVA RE, LE was 20/20: Extrinsic motility, biomicroscopy and IOP were normal. Fundus examination: yellowish deposits well-defined with homogeneous distribution in the retinal pigment epithelium and outer retina. Macular alteration with loss of physiological brightness was found. There were normal vascular attenuation and unaltered optic nerve. AGF showed multiple hypo and hyperfluorescent not exudative spots in the posterior pole and peripheral retina of both eyes and non exudative maculopathy with dystrophic appearance. Perimetric study: bilateral central scotoma and unaltered periphery. OCT: macular thickness decrease and increasing RPE reflectance. Electrophysiological and genetic studies were also done.

**Conclusions:** Clinical association of fundus albipunctatus and subclinical cone dystrophy was presented. Association between this clinical finding and the RDHS5 gene mutation, as described in the literature is discussed.

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**Detached Retina Serous: Key Sign for the Diagnosis of HIV Disease**

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**Introduction:** Syphilitic retinitis is an uncommon manifestation of HIV patients. Here we present the clinical case of a patient who debuted with an anterior and posterior uveitis with serous retinal detachment. The systemic study leaded us to the diagnosis of syphilis and HIV disease.

**Case Presentation:** A 40 year-old male, with no systemic and ocular clinical history of interest, attended the emergency services of our hospital complaining of a sudden monocular visual loss of 48 h of evolution. Best corrected visual acuity was hands movement, the biomicroscopy showed non granulomatous anterior uveitis and the ocular fundus manifested a serous retinal detachment. Biochemical probes confirmed the positivity to syphilis infection and HIV. These data allowed us to diagnose this case and to precisely orienting the uveitis therapeutic protocol, as well as the initiation of systemic treatment for HIV.

**Conclusions:** A systematized study of any inflammatory retinal detachment may be the key for diagnosing and early treating these type of infectious systemic diseases.
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Full-Thickness Macular Hole Associated with Diabetic Foveal Neovascularization

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Introduction: Diabetic foveal neovascularization (DFN) is a rare manifestation of diabetic retinopathy with very few cases reported in the literature. We present a case of unilateral DFN treated with panretinal photocoagulation and repeated intravitreal injections of bevacizumab, complicated by the appearance of a lamellar macular hole (MH) followed by a full thickness MH.

Case Presentation: A 52-year-old woman presented with rapid worsening of her diabetic retinopathy. Best corrected visual acuity (BCVA) was 20/33 in both eyes. Fundus fluorescein angiography of the left eye demonstrated dye leakage from retinal new vessels originated from the perifoveal capillary network. OCT showed a hyper-reflective image attached to the posterior hyaloid and penetrating into the vitreous. Panretinal photocoagulation was performed with regression of neovessels and posterior development of a lamellar MH. BCVA was 20/33 and no intervention was carried out. Two years later a full-thickness MH was evidenced with a BCVA of 20/125. Macular surgery was performed with the closure of the hole and final BCVA of 20/40.

Conclusions: Although the association between DFN and MH has not been previously reported in the literature, this possibility should be considered when evaluating a patient with DFN. Surgical treatment, according our case, provides favorable results.

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Bevacizumab Treatment for Macular Exudation in Juxtapapillary Capillary Hemangioma

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Introduction: We present the long-term results of the treatment with intravitreal (iv) bevacizumab in an exudative juxtapapillary retinal capillary haemangioma (RCH).

Case Presentation: A 72-year-old man with a juxtapapillary RCH and lipid exudation extending into the macula was treated with two monthly iv injections of 125 mg bevacizumab as the sole treatment. Best corrected visual acuity (BCVA), fundus fluorescein angiography (FFA) and optical coherence tomography (OCT) were performed at baseline and six years after treatment.

After treatment a complete regression of lipid exudates and subretinal fluid was observed. BCVA improved from 20/66 to 20/33. FFA showed a reduction of tumor leakage, with a decrease of lesion size.

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Acute Visual Loss after Half Dose Photodynamic Therapy in Chronic Central Serous Chorioretinopathy

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Introduction: We present a case of chronic central serous chorioretinopathy (CCSC) complicated by a severe visual loss due to massive serous neuroretinal detachment (SNRD) after half-dose photodynamic therapy (HD-PDT).

Case Presentation: A 52-year-old woman presented with metamorphopsia and blurred vision in the left eye from two months. Her visual acuity (VA) was 20/20 OD and 20/25 OS. Fluorescein and indocyanine angiography, fundus autofluorescence and optical coherence tomography (OCT) confirmed the diagnosis of CCSC. Given the chronic course of the disease a HD-PDT was applied. Three days after treatment the patient reported severe visual loss (VA = 20/100). OCT revealed a marked increase of the SNRD with a central subfield thickness (CST) of 849 μ and visual field showed a deep and extensive helicoidal scotoma. No treatment was given to the patient. Ten days later the scotoma persisted but CST was reduced to 379 μ. One month after treatment VA improved to 20/33 and CST to 255 μ. Total recovery of vision (VA = 20/20) (CST = 253 μ) occurred at 3 month follow-up.

Conclusion: The use of HD-PDT is not free of possible complications. Massive choroidal non-perfusion and subretinal fluid formation may represent exaggeration of normal events occurring after PDT.

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SD-OCT and Fundus Autofluorescence Findings in a Case of Laser Pointer Induced Maculopathy


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Introduction: To describe the spectral-domain optical coherence tomography and fundus autofluorescence findings in a case of laser pointer induced maculopathy.

Case Presentation: A 15-year-old boy presented with decreased vision after exposure to a green laser pointer. Fundoscopy revealed grey and yellowish round spots in the foveal area of both eyes.

In the last examination, six years after treatment, the tumor remained inactive, the fundus without exudates with a dry macula and the BCVA was 20/25.

Conclusions: Our case underlines that bevacizumab treatment alone can cause a reduction of the tumour-associated exudation, and that these results can be maintained over the long term observation.
eyes (OU). Fundus Autofluorescence (FAF) imaging revealed subtle changes of the normal background macular autofluorescence of the right eye (OD), with hyperautofluorescence dots in the fovea of the left eye (OS). Spectral-domain optical coherence tomography (SD-OCT) showed a variety of changes of the outer retina and retinal pigment epithelium, with disruption of the external limiting membrane, the photoreceptor inner segment-outer segment junction, and the cone outer segment tips in the foveal region OU.

Conclusions: Laser pointers maculopathy may disturb the outer retinal architecture in a manner evident on SD-OCT and FAF resulting in decreased visual acuity. Proper warnings should accompany these devices and access to them by minors should be limited.

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Diagnosis of Cone Dystrophy with Optical Coherence Tomography (OCT)
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Introduction: Macular cone dystrophy is an inherited bilateral disorder whose incidence is 1/400,000. It usually begins between the first and the third decade of life. Optical Coherence Tomography (OCT) is a well-established method of examining the retinal architecture in real time in situ.

Case Presentation: A 28-years-old-woman complained progressive loss of vision and photophobia since childhood. Her vision was better at night and had no color discrimination. Her best corrected visual acuity was 20/30 in both eyes. Fundoscopic examination revealed a pale papilla and vascular narrowing, no pigment in the macula and diffuse alteration of the RPE OU. Automated perimetry revealed a diffuse reduction in sensitivity and macular scotoma in both eyes. Farnsworth showed an unspecific color alteration. The macular OCT scan images showed a clearly bordered area underneath the fovea of loss of the highly back-scattering photoreceptor layer that could correspond with macular cone atrophy in both eyes.

Conclusions: The OCT allows us to know the retina tissue structure with high resolution in vivo. In this degenerative disease permit us to observe a low intraretinal reflectivity in the macular area that is the virtual space who are in the area who leaves the outer photoreceptor segments that never developed.

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Simultaneous Bilateral Central Retinal Vein Occlusion in Von Hippel Lindau Syndrome
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Introduction: To report a case of simultaneous bilateral central retinal vein occlusion in a 48 years old female patient diagnosed with Von Hippel Lindau syndrome (VHL). This condition is characterized by increased susceptibility to kidney, central nervous system and retinal tumors.

Case Presentation: Our patient had no history of interest except insulin-dependent diabetes and two abortions of unknown etiology. Multiple imaging highlighting bilateral renal lesions and at the high heredofamiliar syndrome suspected, genetic study was conducted which confirmed the suspicion of VHL. Interconsultation to ophthalmology was performed to rule out retinal hemangioblastomas although only appeared signs of moderate nonproliferative diabetic retinopathy without macular edema. After genetic confirmation and compatible of bilateral renal carcinoma images, bilateral nephrectomy was performed. At 3 months postoperatively, the patient had bilateral sudden loss of vision caused by simultaneous bilateral central retinal vein occlusion with cystoid macular edema pending anti-VEGF treatment. After clinical follow up hypertension or blood disorders were discarded.

Conclusions: Simultaneous bilateral central retinal vein occlusion is a rare entity and is usually associated with hyperviscosity syndromes or hypertension. In the absence of both factors might be some vascular or inflammatory disorder not well known to justify the case.

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Gitelman Disease and Sclerochoroidal Calcification
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Introduction: Sclerochoroidal calcification (SCC) is commonly seen in middle-age asymptomatic individuals. Although most cases are idiopathic, an underlying systemic disorder may be present and should be ruled out.

Case Presentation: A 42 years-old woman was referred for evaluating a “bilateral chorioretinal mass”. Her visual acuity was 20/20 in both eyes. The dilated fundus examination (DFE) showed a yellowish irregular lesion with sharply defined borders in the superior margin of superotemporal arcades. Spectral domain optical coherence tomography (SD-OCT) revealed compriosis of the choroidal tissue by an inwards bulging scleral lesion without any sign of exudation and preservation of the neurosensel retina. She was diagnosed of Gitelman syndrome on the basis of hypokalemic metabolic alkalosis with hypocalciuria and hypomagnesemia.
Conclusion: In the presence of SCC is mandatory to rule out underlying systemic diseases as these might be potentially treatable. SCC exhibits typical DFE and SD-OCT findings. It does not require any further intervention. The etiopathogenesis of SCC remains unclear.

Peripheral Retinal Ischemia in Waldenström’s Macroglobulinemia
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Introduction: Waldenström’s macroglobulinemia (WM), a non-Hodgkin’s B-cell lymphoplasmocytic lymphoma presents a production of monoclonal IgM and infiltration of bone marrow. Hyperviscosity syndrome is caused by the presence of abnormal plasma proteins. The signs of retinopathy are venous dilation, retinal hemorrhages, segmentation and tortuosity, initially in the periphery and in advanced cases in central retina with or without papilledema.

Case Presentation: A 53 years-old man who consulted for painless, progressive loss of vision in both eyes associated with epistaxis, fatigue and weight loss. There was no significant past ocular or medical history. In view of the findings on physical and ophthalmic examination, we asked for an urgent interconsultation with the department of hematology that diagnosed Waldenström macroglobulinemia and treated the patient with plasmapheresis. Besides characteristic signs of hyperviscosity in fundoscopic examination, angiofluoresceinography showed areas of ischemia in peripheral retina. Finally, the patient referred partial improvement of visual acuity persisting retinal hemorrhages.

Conclusions: Although incidence rate of this disease is low, the exhaustive examination of the central and peripheral retina by funduscopy and fluorescein angiography is important in order to detect areas of peripheral ischemia. Waldenström macroglobulinemia should form part of the differential diagnosis of peripheral retinal ischemia.

Autologous Fibrin for the Treatment of Myopic Foveoschisis with a Macular Hole
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Introduction: To report a case in which we used peeling the internal limiting membrane (ILM) and autologous fibrin for the treatment of myopic foveoschisis associated with poor prognosis retinal detachment and a macular hole.

Case Presentation: A 76 years old woman with high myopia presented to our Service with a macular hole associated with a retinal detachment of several months evolution. A pars plana vitrectomy was carried out through microsclerotomies. Posterior hyaloid membrane and the ILM were peeled up to the temporal vascular arcades with the aid of intravitreal injections of triamcinolone acetonide and brilliant blue. Three drops of autologous fibrin (obtained with the Vivostat® System) were applied on the central macular area at the end of the surgery. One day after surgery, the macula was reattached and the hole was closed, as judged by OCT. The functional outcome was also adequate, as the best corrected visual acuity increased from 0.16 preoperatively to 0.3 and 0.4 at 3 and 12 months after surgery. Fibrin was fully reabsorbed by 10 days after the surgery.

Conclusions: Autologous fibrin may contribute to macular hole closure and may improve the anatomical and functional results in macular holes with poor prognosis.