23rd Meeting of the
European Association for the Study of Diabetes Eye Complications Study Group (EASDec)

Barcelona, Spain
May 23–25, 2013

Free Paper Sessions

OVEREXPRESSION OF HEMOPEXIN IN THE DIABETIC EYE: A NEW PATHOGENIC CANDIDATE FOR DIABETIC MACULAR EDEMA

M. Garcia-Ramírez, C. Hernández, R. Simó
Diabetes and Metabolism Research Unit. Vall d'Hebron Research Institute. Universitat Autònoma de Barcelona, Spain

Introduction. Case-control study and experimental study.
Purpose. Hemopexin is a well-recognized permeability factor in the kidney but its potential role in blood-retinal-barrier (BRB) breakdown has not been explored. The main aims of this study were: 1) To determine hemopexin expression in the retina and its content in the vitreous fluid from diabetic patients with diabetic macular edema (DME) and non-diabetic patients; 2) To evaluate the effect of hemopexin on BRB permeability; 3) To determine whether dexamethasone prevents an eventual hemopexin-induced hyperpermeability.
Methods. Biological material: 1) Retinas from 10 diabetic donors with non-proliferative retinopathy (NPDR) and from 10 non-diabetic donors. 2) Vitreous fluid from 14 patients with DME and 14 non-diabetic patients. Hemopexin and LRP1 mRNA levels were measured by quantitative RT-PCR and hemopexin concentrations by ELISA. The effect of hemopexin on permeability in culture was evaluated by fluorescein isothiocyanate dextran movements in APRE-19 cells and bovine retinal endothelial cells. The experiments were repeated in the presence of hemopexin neutralizing antibodies and dexamethasone.
Results. A higher expression of hemopexin was detected in the RPE from diabetic patients in comparison with non-diabetic controls. Intravitreal hemopexin concentration was higher in patients with DME than in non-diabetic subjects. Hemopexin significantly increased permeability in ARPE-19 cells which was prevented by both hemopexin neutralizing antibodies and dexamethasone.
Conclusions. Hemopexin is overexpressed in the RPE of diabetic patients with DME and induces the breakdown of RPE cells *in vitro*. Dexamethasone was able to prevent hemopexin-induced hyperpermeability. Our results suggest that hemopexin can be considered a new pathogenic candidate for DME.

DEVELOPMENT OF A NEW BLOOD-RETINAL BARRIER MODEL *IN VITRO*

A. Van der Wijk[1], J. Wisniewska-Kruk[1], I. Klaassen[1], R. O. Schlingemann[1,2]
[1]Ocular Angiogenesis Group, Departments of Ophthalmology and Cell Biology and Histology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; [2]Department of Clinical and Molecular Ophthalmogenetics, Netherlands Institute for Neuroscience, Royal Netherlands Academy of Arts and Science (KNAW), Amsterdam, The Netherlands

Introduction. We developed a new *in vitro* blood-retinal barrier (BRB) model by co-culturing endothelial cells, pericytes and astrocytes in a Transwell filter system.
Purpose. The loss of blood-retinal barrier (BRB) properties is an important feature in the pathology of vision-threatening diabetic retinopathy (DR). Despite increased knowledge about BRB physiology, little is known about the cellular mechanism underlying its dysfunction in DR and other pathological conditions.
Methods. Primary Bovine Retinal Endothelial Cells (BRECs) were co-cultured with bovine pericytes and primary rat astrocytes on Transwell filters in different combinations. Various culture media were tested in order to obtain the most optimal culture conditions. The BRB properties were assessed by measurement of trans electric endothelial resistance (TEER) and the expression of endothelial junction proteins was visualized by immunocytochemistry and examined with confocal microscopy.
Results. BRECs co-cultured with rat astrocytes and bovine pericytes in medium supplemented with cAMP and RO-20-1724 expressed VE-Cadherin, ZO-1, Claudin-5 and Occludin protein and showed the highest TEER-value. Moreover, the high barrier properties of this model were confirmed by permeability analysis using fluorescently labelled dextrans of different sizes.
Conclusions. We developed a new co-culture BRB model *in vitro* that is a great and unique tool to study and understand in more detail the mechanisms that cause the loss of BRB *in vivo*. This may open novel therapeutic avenues for testing more efficient treatment of DR and other retinal disorders.

MOLECULAR CHARACTERIZATION OF THE INNER BLOOD-RETINAL BARRIER IN THE AKIMBA MOUSE, A NEW MODEL OF DIABETIC RETINOPATHY

I. Klaassen[1], J. Wisniewska-Kruk[1], I. M. C. Vogels[1], C. M. Lai[3], C. J. F. Van Noorden[1], R. O. Schlingemann[1,2], E. P. Rakoczy[3]

[1]Ocular Angiogenesis Group, Departments of Ophthalmology and Cell Biology and Histology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; [2]Department of Clinical and Molecular Ophthalmogenetics, Netherlands Institute for Neuroscience, Royal Netherlands Academy of Arts and Science (KNAW), Amsterdam, The Netherlands; [3]Department of Molecular Ophthalmology, Lions Eye Institute, Nedlands, 6009 Western Australia

Introduction. To study the combination of hyperglycaemia and high intraretinal VEGF on BRB integrity in diabetic retinopathy, the Akimba mouse was generated by crossing the Akita mouse (hyperglycemia by insulin 2 gene mutation) with the Kimba mouse (intraretinal overexpression of hVEGF).

Purpose. Breakdown of the inner endothelial blood-retinal barrier (BRB), as occurs in diabetic retinopathy, results in vasogenic edema and neural tissue damage, causing loss of vision. One of the limitations of research into diabetic retinopathy is the lack of suitable animal models. The recent Akimba mouse model is the first model that shows the full extent of clinical signs of diabetic retinopathy, including retinal edema.

Methods. BRB loss, represented by increased vascular permeability, was assessed by fluorescein angiography in 8-9 weeks old wild type, Akita, Kimba and Akimba mice (n=7 per group). Next, the eyes were collected and RNA from the whole retina was isolated. Transcription levels were determined by real-time quantitative RT-PCR and proteins were visualized by immunohistochemistry on cryosections. Transcription levels of Kimba and Akimba mice were correlated to the degree of fluorescein leakage, determined by two masked ophthalmologists based on 4-grade scale.

Results. In Kimba and Akimba mice, a respective 32- and 16-fold increase in Plvap mRNA levels was found, and Plvap levels were significantly correlated to the degree of fluorescein leakage in these two mouse models. Despite fluorescein leakage in retinas of Kimba mice, no significant alterations in endothelial junctions or pericytes were observed. In contrast, the Akimba was characterized by pericyte drop out, vasoregression and decreased gene expression of endothelial junction proteins.

Conclusions. Although the Kimba mouse displays many features of clinical DR, the addition of hyperglycaemia in the Akimba mouse seems to exaggerate the vascular changes seen in the Kimba mouse. In both models Plvap was highly increased and statistically significant related to the degree of fluorescein leakage, suggesting an important role for Plvap in pathological retinal vascular leakage.

THE ROLE OF PLASMALEMMA VESICLE-ASSOCIATED PROTEIN IN BLOOD RETINAL BARRIER PERMEABILITY

J. Wisniewska-Kruk[1], I. Klaassen[1], I. M. C. Vogels[1], C. J. F. Van Noorden[1], R. O. Schlingemann[1,2]

[1]Ocular Angiogenesis Group, Departments of Ophthalmology and Cell Biology and Histology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; [2]Department of Clinical and Molecular Ophthalmogenetics, Netherlands Institute for Neuroscience, Royal Netherlands Academy of Arts and Science (KNAW), Amsterdam, The Netherlands

Introduction. Loss of blood-retinal barrier (BRB) properties is an important feature in the pathology of diabetic retinopathy (DR). However, little is known about the cellular mechanism underlying its dysfunction in pathological conditions. Up to date, two mechanisms have been proposed to be involved in BRB disruption in DR: paracellular transport due to changes of tight junction integrity and increased transcellular transport mediated by caveolae. Plasmalemma vesicle-associated protein (PLVAP), an endothelial specific protein which is believed to form stomatal diaphragms on caveolae, was previously found to be increased in clinical and experimental diabetes and induced by VEGF *in vitro* and *in vivo*.

Purpose. To investigate whether PLVAP is necessary for BRB integrity, we knocked-down the PLVAP gene in an *in vitro* BRB model and in an *in vivo* mouse model and measured the effect on paracellular and transcellular BRB permeability.

Methods. Primary bovine retinal endothelial cells were seeded on Transwell filters and PLVAP mRNA was silenced by a lentiviral approach. The effect on BRB integrity was assessed by measurement of transendothelial resistance (TEER), permeability with labelled molecules of different sizes, and immunohistochemical staining of tight junction proteins. Furthermore, real-time qPCR and cell-based ELISA were used to investigate mRNA and protein levels of molecules involved in paracellular and transcellular transport of the BRB. The oxygen induced retinopathy model was used to induce BRB permeability. PLVAP was silenced by intraocular injections of siRNA. Retinal leakage was measured by fluorescent labeled dextrans after intracardial injections.

Results. After induction with VEGF, TEER-values dropped, permeability increased and tight junction staining was decreased in the BRB model. Inhibition of PLVAP mRNA could prevent these effects in the presence of VEGF and showed differential effects on mRNA and protein levels of the various BRB molecules. Similarly, silencing of Plvap *in vivo* resulted in decreased permeability of retinal vessels for 70 kDa dextrans.

Conclusions. Our data indicate that inhibition of PLVAP may prevent VEGF-induced permeability in the BRB. This suggests that transcellular transport plays a significant role in BRB disruption and may be an interesting target in the treatment of DME.

AQUEOUS HUMOR BIOMARKERS OF RETINA MACROGLIA ACTIVATION IN DIABETIC PATIENTS

S. Vujosevic[1], M. Berton[1], S. Bini[1], A. Micera[2], G. Esposito[2], O. Kotsafti[2], E. Midena[1,2]

[1]Department of Ophthalmology University of Padova, Padova, Italy; [2]GB Bietti Foundation, IRCCS, Roma, Italy

Introduction. Cross-sectional, comparative case series.

Purpose. To identify early biomarkers of retina macroglia activation in diabetic patients without and with non proliferative diabetic retinopathy (DR).

Methods. During cataract surgery, 55 patients' aqueous humor (AH) samples were collected as follows: 18 healthy subjects, 19 diabetic patients without DR and 18 diabetic patients with non proliferative diabetic retinopathy (9 without macular edema-no DME and 9 with DME). Before surgery, full ophthalmic examination and Spectral-Domain Optical Coherence Tomography (SD-OCT) (Spectralis HRA+OCT, Heildeberg Engineering) were performed in all eyes. AH was sampled in all eyes using a 30 gauge needle through a peripheral clear cornea approach, before cataract extraction. The samples were analyzed for the quantification of glial fibrillary acidic protein (GFAP), aquaporine 1 (AQP1) and AQP4 as biomarkers of retinal macroglial activity, by ELISA. ANOVA analysis followed by Tukey-Kramer post-hoc test was applied.

Results. There was not significant difference in the age among the four groups. Mean concentration of GFAP, AQP1 and AQP4 significantly increased in diabetic eyes versus controls (324.4±262.5 pg/µg vs 182.3±114.4 pg/µg for GFAP; 105.7±15.7 pg/µg vs 50.9±20.4 pg/µg

for AQP1; and 852+103.2 pg/µg vs 33.6±21.2 pg/µg for AQP4; p<0.005, for each comparison). GFAP showed an approximate 0.8 fold increase, AQP1 1.1 fold increase, whereas AQP4 about 24 folds increase in diabetic patients versus controls. When DR-no DME eyes and DR-DME eyes were separately evaluated, there was a significant decrease in GFAP, AQP1 e AQPR in DR-DME eyes versus DR-noDME eyes, (Tukey Kramer post hoc p<0.05). GFAP and AQP1 showed even a slight, non significant, fold decrease versus controls. AQP4/AQP1 concentration showed weak and non significant correlation (Tau=0.21, p=0.3) between these biomarkers, despite increasing trend.

Conclusions. GFAP, AQP1 and AQP4 are known as biomarkers of retinal macroglia activity. All these biomarkers are significantly increased in human eyes with diabetes, confirming that retinal glia is a key actor in this disorder. The decrease of these biomarkers in eyes with DME probably represents a sign of Müller cells degeneration.

RETINAL INFLAMMATION BIOMARKERS IN PATIENTS WITH DIABETIC RETINOPATHY

E. Midena[1,2], G. Midena[1], S. Bini[1], M. Berton[1], R. Parrozzani[2], O. Kotsafti[2], S. Vujosevic[1]

[1]Department of Ophthalmology University of Padova, Padova, Italy; [2]GB Bietti Foundation, IRCCS, Roma, Italy

Introduction. Cross-sectional, comparative case-series.

Purpose. To evaluate *in vivo* biomarkers of retinal inflammation (microglia activity and aqueous humor cytokines) in patients with diabetic retinopathy (DR).

Methods. 30 diabetic patients (10 with mild non proliferative DR (Mi-DR), 10 with moderate DR (Mo-DR) and 10 with severe DR (Se-DR) and 10 normal subjects needing cataract surgery were evaluated. All patients underwent full ophthalmic examination including spectral domain-OCT (SD-OCT) and aqueous humor (AH) sample collection. Proliferative DR, previous laser photocoagulation, intraocular surgery or intravitreal injection, glaucoma or ocular hypertension and neurodegenerative diseases were the main exclusion criteria. One eye of each subject was used for statistical analysis. After segmentation of retinal layers by SD-OCT, retinal images were analyzed for reflectivity changes (hyperreflectivity spots) at the level of: internal limiting membrane + retinal nerve fiber layer (ILM+RNFL), inner nuclear layer+outer plexiform layer (INL+OPL) and outer nuclear layer. All examinations were performed twice, by two independent masked graders. Protein array for 40 inflammatory proteins was performed.

Results. No statistically significant differences were found for age among all groups and for glycemic control between diabetic groups. The inter-grader agreement was at least substantial for all measurements. In DR eyes hyperreflective spots were systematically detected at the level of ILM+RNFL (75% vs 87% vs 98% in Mi-DR vs Mo-DR vs Se-DR, respectively; p<0.0001) and their expression significantly progressed toward outer retinal layers with the progression of DR steps (p<0.005 for INL+OPL in the three groups; p<0.001 for ONL in the three DR groups). Following cytokines were increased in diabetic patients' AH compared to healthy subjects: IFNy, IL-1a, IL-1b, IL-3, IL-4, IL-10, IL-11, IL-17, TNF-α, TNF-β, MCP1, MCP2, Eotaxin, Eotaxin 2, RANTES, sTNFRII, GM-CSF, IP-10, MIP1a, MIP1b.

Conclusions. The presence of retinal discrete microaggregates, documented as hyperreflective spots, in areas corresponding to microglial cells may represent an *in vivo* biomarker of retinal microglial activation in diabetes. These correlate well with aqueous humor cytokine increase in DR patients. Retinal neuroinflammatory biomarkers could provide useful therapeutic targets for DR prevention and therapy.

MICROANEURYSM TURNOVER PREDICTS RISK OF DEVELOPMENT OF CLINICALLY SIGNIFICANT DIABETIC MACULAR EDEMA

L. Ribeiro, S. Nunes, J. Cunha-Vaz

AIBILI - Association for Innovation and Biomedical Research on Light and Image, Coimbra, Portugal

Introduction. Prospective, monocenter, observational study to follow eyes/patients with diabetes type 2 and nonproliferative diabetic retinopathy (NPDR) (ETDRS levels 20 and 35) with no prior laser treatment for two years or until development of clinically significant macular edema (CSME). (Clinical Trial registration: NCT00763802, ClinicalTrials.gov)

Purpose. To examine the relationship between microaneurysm (MA) turnover using automated analysis of fundus photographs (RetmarkerDR) and development of CSME in NPDR.

Methods. 410 patients, one eye per patient, fulfilled the inclusion/exclusion criteria and were included in the study. Ophthalmologic examinations including BCVA, fundus photography and optical coherence tomography (OCT) were performed at baseline, six-month and at the last study visit (24-month or before laser treatment).

Results. 348 eyes performed the 24-month visit or developed CSME. Of these 348 eyes 26 developed CSME. MA turnover (i.e. the sum of the MA formation and disappearance rates) computed during the first 6 months of follow-up were found to be independently predictive factors for development of CSME. MA turnover was 11.2+/-11.2 in the 26 eyes that developed CSME and 5.0+/-5.2 in the remaining 322 (p<0.001). Higher MA turnover values correlated with earlier development of CSME.

Conclusions. MA turnover calculated with the RetmarkerDR predicts development of CSME in eyes with NPDR.

MICROANEURYSM-COUNT AS A PREDICTOR OF PROGRESSION OF RETINOPATHY IN PATIENTS WITH TYPE 1 DIABETES?

M. L. Rasmussen[1,2], R. B. Pedersen[1], T. Peto[2], J. Grauslund[1]

[1]Department of Ophthalmolgy, Odense University Hospital, University of Southern Denmark, Denmark; [2]NIHR Biomedical Research Centre, Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London, UK

Introduction. A 16-year prospective population-based cohort study of Danish patients with Type 1 diabetes.

Purpose. Early predictors of diabetic retinopathy (DR) are needed in order to identify patients with type 1 diabetes at risk of loosing sight. The aim was to investigate microaneurysm (MA)-count as a predictor of progression.

Methods. A total of 139 patients were examined at baseline in 1995 and at follow-up in 2011. At baseline HbA1c, albumin excretion, blood pressure were measured and BMI was calculated. Two-field (1995) and seven-field (2011) fundus photographs were taken. MAs at baseline were counted. DR was graded in accordance to ETDRS allowing for non-standard photography in 1995. Only participants with either no DR or MAs only in at least one eye at baseline (ETDRS level 10/10, 10/20, 20/20 or 20/35) were included. Patients with level 20/35 only participated with the 'level 20'-eye. For analysis the worst eye at follow-up was used. Progression to proliferative DR (PDR) was defined as level 61 or above in 2011. A logistic regression was made for MA-count as predictor of progression to PDR, adjusting for potential baseline risk factors: sex, age, duration of diabetes, DR at baseline, blood pressure, nephropathy and BMI.

Results. Altogether 227 eyes of 120 patients were eligible for inclusion. Of these, 122 eyes had ETDRS level 10 and 105 had level 20. In

the latter group, median MA-count was 2 (range 1-21). In a multivariate logistic regression model progression to PDR was predicted by MA-count (odds ratio[OR]: 1.58 per MA, 95% CI 1.02-2.43; p=0.039). The level of DR at follow-up ranged from 10 to 85, with 60.8% being at or below level 35. The 16-year incidence of DR and PDR was 91.5% and 17.0%, respectively. Only 4 patients did not develop DR in 16 years and another 4 remained stable.

Conclusions. Based on our findings, MA-count can be included in clinical assessment and may serve as a predictor of progression to PDR in patients with type 1 diabetes. MA-count is time-consuming so development in (semi) automated image analysis will be necessary for inclusion in clinical practice.

PATTERNS OF PROGRESSION IN DIABETIC RETINOPATHY. CORRELATION BETWEEN PHENOTYPES AND GENOTYPES

S. Nunes[1,2,3,4], C. Lobo[1,2,3], L. Ribeiro[1,2], I. Pires[1,2,3], M. J. Simões[4], C. Faro[4], J. Cunha-Vaz[1,2]

[1]AIBILI – Association for Innovation and Biomedical Research on Light and Image, Coimbra, Portugal; [2]IBILI / FMUC – Institute of Biomedical Research on Light and Image, Faculty of Medicine of the University of Coimbra, Coimbra, Portugal; [3]CRIO / CHUC – Centro de Responsabilidade Integrado de Oftalmologia, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; [4]BIOCANT - Biotechnology Innovation Center, Cantanhede, Portugal

Introduction. Observational prospective.

Purpose. To establish a correlation between phenotypes of non-proliferative diabetic retinopathy (NPDR) progression (phenotypes A, B and C) and different candidate genes in patients with Type 2 diabetes.

Methods. A population of 307 diabetic patients with NPDR, followed-up during a 2 years prospective study was classified in 3 different phenotypes of DR progression based on non-invasive methods, Color Fundus Photography (CFP) to assess microaneurysm turnover (MAT) and Optical Coherence Tomography (OCT) to measure 9≤Retinal Thickness (RT). Phenotype A was considered for MAT & Normal RT; 9≤Phenotype B for MAT & Increased RT; and Phenotype C for MAFR>9 & Normal or Increased RT. Twenty one (21) patients/eyes developed during the 2-year study clinically significant macular edema (CSME), 14 from Phenotype C, 5 from Phenotype B and 2 from Phenotype A. Eleven genes were selected from a list of candidate genes (ACE, AGER, AKR1B1, ICAM1, MTHFR, NOS-1, NOS-3, PPARGC1A, TGFB1, TNF-α, and VEGFA). Correlations between genotypes and phenotypes were tested using logistic regression models, adjusted for age and sex.

Results. The distribution for the 3 phenotypes was respectively 54.1%, 23.4% and 22.5%. Statistically significant differences between phenotypes were found for NOS1 (rs1552228, P=0.015). When considering patients that developed CSME, statistically significant differences were found for ACE (rs35865660, P=0.049) and MTHFR (rs7533315, P=0.013).

Conclusions. ACE, NOS1 and MTHFR were found to be associated with different phenotypes of DR progression and development of CSME. The identification of these phenotypes-genotypes correlations opens new perspectives for the management and the treatment of DR in patients with Type-2 diabetes.

OBSTRUCTIVE SLEEP APNOEA IS ASSOCIATED WITH SIGHT THREATENING RETINOPATHY AND PREDICTS THE DEVELOPMENT OF PREPROLIFERATIVE AND PROLIFERATIVE RETINOPATHY IN PATIENTS WITH TYPE 2 DIABETES: A LONGITUDINAL ANALYSIS

A. Tahrani[1,2], P. Dodson[2,3,4], A. Ali[5], Q. Altaf[2], H. Wharton[2,3], N. T. Raymond[6], M. J. Stevens[1,2]

[1]Centre of Endocrinology, Diabetes and Metabolism, University of Birmingham, Birmingham, UK; [2]Department of Diabetes and Endocrinology, Heart of England NHS Foundation Trust, Birmingham, UK; [3]Heart of England Retinal Screening Centre, Heart of England NHS Foundation Trust, Birmingham, UK; [4]School of Life and Health Sciences, Aston University, Birmingham, UK; [5]Department of Respiratory Medicine, University Hospital of Coventry and Warwickshire, Coventry, UK; [6]Division of Health Sciences, Warwick Medical School, University of Warwick, Coventry, UK

Introduction. An observational longitudinal study.

Purpose. Assess the relationship between obstructive sleep apnoea (OSA) and DR cross-sectionally and longitudinally.

Methods. Adults with Type 2 diabetes mellitus (T2DM), who were recruited from a hospital-based diabetes clinic in the UK. Patients with pre-existing OSA, end-stage renal disease and non-diabetic retinopathy were excluded. OSA (apnoea hypopnea index ≥ 5 events/hour) was assessed by a single overnight home-based cardio-respiratory study (Alice PDX, Philips Respironics, USA). DR was assessed using retinal images between 2007 and 2012. Sight threatening diabetic retinopathy (STDR) was defined as presence of pre-proliferative or proliferative DR, maculopathy or photocoagulation. Advanced DR was defined as pre-proliferative or proliferative DR.

Results. 199 patients were included (57.3% (n=114) men, 47.7% (n=95) White Europeans). STDR and OSA prevalence were 38.7% (n=77) and 62.8% respectively.

At baseline. STR prevalence was higher in patients with OSA (OSA+) compared to those without OSA (OSA-) [48.8% n=61 vs. 21.6% n=16, p<0.001]. After adjustment for confounders, OSA remained independently associated with STR (OR 3.7, 95% CI 1.6-8.9, p=0.006), maculopathy (OR 4.5, 95% CI 1.8-11.4, p=0.002) and advanced DR (OR 3.9, 95% CI 1.02-15.3, p=0.047). Mild and moderate to severe OSA were independently associated with STR and maculopathy and only moderate to severe OSA was associated with advanced DR following adjustment for confounders.

Longitudinally. Over the follow-up period of (4.4±1 years), more OSA+ patients progressed from no or background DR to advanced DR (15.3% (n=17) vs. 3% (n=2), p=0.01). OSA was an independent predictor of advanced DR development after adjustment for confounders (OR 6.6, 95% CI 1.2-35.1, p=0.03). OSA did not predict the development of maculopathy.

Conclusions. OSA is independently associated with STR and predicts the development of preproliferative and proliferative DR. Interventional studies are needed to assess the impact of OSA treatment on DR.

LOSS OF PROTEIN TYROSINE PHOSPHATASE 1B INCREASES IGF-I RECEPTOR TYROSINE PHOSPHORYLATION BUT DOES NOT RESCUE RETINAL DEFECTS IN IRS2-DEFICIENT MICE

A. M. Valverde[1,2] J. Revuelta-Cervantes[1,2], L. Menes[2,3], A. González-Rodriguez[1,2], V. Pardo[1,2], P. de la Villa[4], D. J. Burks[2,3], A. I. Arroba[1,2]
[1]Institute of Biomedicine Alberto Sols (CSIC/UAM), C/ Arturo Duperier 4, 28029 Madrid, Spain; [2]Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), ISCIII, Spain; [3]Research Center Príncipe Felipe, Avda. Autopista del Saler, 16, 46012 Valencia, Spain; [4]Department of Physiology, University of Alcalá, 28871 Alcalá de Henares, Spain

Introduction. This study was designed to evaluate role of protein tyrosine phosphatase 1B in mediating IGF-IR/Akt signaling cascade in the retina.
Purpose. Our goal was to analyze IGF-IR-mediated survival signaling and visual function in PTP1B-deficient (PTP1B-/-) mice and double mutant mice deficient in both PTP1B and IRS2 (IRS2-/-/PTP1B-/-) in comparison to wild-type and IRS2-deficient (IRS2-/-) mice.
Methods. IGF-IR tyrosine phosphorylation and Akt serine 473 phosphorylation were analyzed by western blot in organotypic retinal explants stimulated with IGF-I. Immunohistochemistry was used to evaluate retinal structure preservation in mice at 9 weeks. Visual function was evaluated by Electroretinographic (ERG) recording in mice at 5 and 9 weeks.
Results. IGF-IR tyrosine phosphorylation and Akt serine phosphorylation increased in retinal explants of wild-type mice stimulated with IGF-I in a dose-dependent manner. In PTP1B-/- retinal explants, these responses were enhanced. Conversely, in retinas from IRS2-/- mice levels of PTP1B were increased and IGF-IR-mediated Akt phosphorylation decreased as compared to the wild-type control. PTP1B deletion in IRS2-/- mice also enhanced IGF-IR tyrosine phosphorylation but, unexpectedly, the response to IGF-I in Akt phosphorylation remained decreased as observed in IRS2-/- mice. PTEN was increased in IRS2-/- retinas and remained elevated in IRS2-/-/PTP1B-/- mice. Histological evaluation revealed alterations in various structures of the retina in both IRS2-/- and IRS2-/-/PTP1B-/- mice, specifically in the outer nuclear layer (ONL) and retinal outer segments (ROS). ERG analysis revealed that PTP1B deficiency did not restore normal visual function in IRS2-/- mice.
Conclusions. Although PTP1B deficiency increased IGF-IR tyrosine phosphorylation in retinal explants of IRS2-/- mice, it was unable to restore Akt phosphorylation probably due to elevated PTEN levels. Consequently, structural and functional visual defects of IRS2-/- mice were not improved.

MORPHOLOGICAL AND FUNCTIONAL CHARACTERIZATION OF RETINAL NEURODEGENERATION IN DB/DB MICE

P. Bogdanov[1,2], L. Corraliza[1,2], A. R. Rodrigues-Carvalho[3], J. Garcia-Arumí[3], R. Simó[1,2], C. Hernández[1,2]
[1]Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), ISCIII, Spain; [2]Diabetes and Metabolism Research Unit, Vall d'Hebron Research Institute. Universitat Autònoma de Barcelona, Spain; [3]Ophthalmologic Research Laboratory, Vall d'Hebron Research Institute. Universitat Autònoma de Barcelona, Spain

Introduction. Experimental case-control study.
Purpose. The aim of the present study was to characterise the sequential events that are taking place in retinal neurodegeneration in a murine model that carries a mutation in the leptin receptor gene and develops spontaneous type 2 diabetes (db/db mice.
Methods. A total of 72 C57BL/6 mice were divided into two groups: diabetic (db/db) and non-diabetic (db/+). To assess the chronological sequence of the abnormalities the analysis was performed at different evolutionary stages (8, 16 and 24 weeks). The retinas were evaluated in terms of morphological (total retinal thickness, thickness of retinal layers and ganglion cell count) and functional abnormalities assessed by electroretinography (ERG). In addition, histological markers of neurodegeneration (glial activation and apoptosis) were evaluated. Moreover, GAD-65/67 was determined by immunohistochemistry. Furthermore, to examine the effect of lowering blood glucose levels on retinal neurodegeneration 8 db/db mice 8 weeks old received subcutaneous injections of liraglutide (400 µg/kg/day) for 15 days. Eight db/db treated with vehicle served as control group. Statistical analysis: Comparisons of continuous variables between diabetic and non-diabetic mice were performed using the unpaired Student *t*-test.
Results. Total retinal thickness in both central and peripheral retina was significantly decreased in diabetic mice in comparison with non diabetic mice at 16 and 24 weeks. Glial activation was higher in diabetic than in controls in all the stages ($p<0.01$). In addition, a progressive loss of ganglion cells was detected in diabetic mice. All these histological hallmarks of neurodegeneration were less pronounced at week 8 than at week 16 and 24. Likewise, GAD 65/67 was significantly reduced in diabetic mice in comparison with the controls. At 16 and 24 weeks the diabetes significantly delayed OPs and b-wave implicit time and reduced OPs and b wave amplitude vs. control mice as observed in human patients. Finally, morphological and ERG abnormalities were prevented by lowering blood glucose levels.
Conclusions. Diabetes rather than genetic factors is the primary cause of neurodegeneration in db/db mice which reproduce the features of the neurodegenerative process that occurs in the human diabetic eye. Our results suggest that db/db mice are an appropriate murine model for investigating the underlying mechanisms of diabetes-induced retinal neurodegeneration and for testing neuroprotective drugs.

MESENCHYMAL STEM CELL-DERIVED MICROVESICLES INDUCE PERICYTE DESTABILIZATION IN DIABETIC-LIKE CONDITIONS

E. Beltramo, E. Berrone, T. Lopatina, M. Porta
Department of Medical Sciences, University of Turin, Italy

Introduction. During angiogenesis, the stable association between pericytes and endothelial cells (EC) in pre-existing vasculature is disrupted, leading to EC proliferation. Recent findings suggest the existence of an interest way of intercellular communication, where the 'units' of information are microvesicles (MVS), containing biologically active proteins and RNA, that may promote phenotypic changes in cells. MVS derived from injured cells may induce dedifferentiation of pericytes allowing their detachment from vessels. The literature on the molecular interactions between EC-derived MVS and pericytes for the angiogenic switch is significant, but little is known about mesenchymal stem cells (MSC), a self-renewing cells that can be found in almost tissues, to activate pericytes.
Purpose. Our study aimed at evaluating if MVS produced by MSC in hypoxia and/or hyperglycaemic-like conditions was able to induce pericyte detachment and influence their survival.
Methods. MVS were extracted from the supernatant of MSC cultured in hypoxic and/or high glucose conditions and added to pericytes (HRP) cultured in physiological conditions both on plastic and EC-produced extracellular matrix. We evaluated HRP detachment after 2, 4, 6 and 24 hrs of exposure to MVS by cell counts, as well as viability, cytotoxicity and apoptosis by an ELISA/fluorimetric assay. Motility and cell modifications were analyzed by a MicroImage analysis system.

Results. The number of HRP still attached decreased in a time-dependent way after addition to the culture medium of MVS obtained in all the above-described conditions. Pericyte destabilization was increased by the addition of MVS obtained in both hypoxic and/or high glucose conditions. Apoptosis and viability did not change in all experimental conditions, while HRP cytotoxicity was significantly increased by the addition of MVS produced in hypoxia and high glucose. Changes in cell motility were observed in the presence of MVS, together with an evident cell contraction.
Conclusions. We can conclude that MSC-derived MVS induce HRP detachment, a possible indicator of destabilization. Since this effect is increased by culturing MSC in hypoxic and/or hyperglycaemic-like conditions, it is possible that diabetic-like conditions may influence vessel stabilization during angiogenesis.

VITREOUS TIMP-1 LEVELS ASSOCIATE WITH NEOVASCULARIZATION AND TGF-2 LEVELS IN THE CLINICAL COURSE OF PROLIFERATIVE DIABETIC RETINOPATHY

I. Vogels[1,2], R. J. Van Geest[1,2], I. Klaassen[1,2], S. Y. Lesnik-Oberstein[2], R. Goldschmeding[3], C. J. F. Van Noorden[1,2], R. O. Schlingemann[1,2,4]
[1]Ocular Angiogenesis Group, Departments of Ophthalmology and Cell Biology and Histology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; [2]Department of Ophthalmology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; [3]Department of Pathology, University Medical Center Utrecht, Utrecht, The Netherlands; [4]Department of Clinical and Molecular Ophthalmogenetics, Netherlands Institute for Neuroscience, Royal Netherlands Academy of Arts and Science (KNAW), Amsterdam, The Netherlands

Introduction. Vitreous samples of a prospective cohort of patients with PDR, diabetes without PDR and non-diabetic controls were analysed and correlated to the clinical degree of neovascularisation and fibrosis.
Purpose. In proliferative diabetic retinopathy (PDR), vascular endothelial growth factor (VEGF) and connective tissue growth factor (CTGF) cause blindness by neovascularization and subsequent fibrosis. This angio-fibrotic switch is associated with a shift in the balance between vitreous levels of CTGF and VEGF in the eye. Here, we investigated the possible involvement of other important mediators of fibrosis, tissue inhibitor of matrix metalloproteinases (TIMP)-1 and transforming growth factor (TGF)-β2 in these stages of PDR.
Methods. TIMP-1, activated TGF-β2, CTGF and VEGF levels were measured by ELISA in 78 vitreous samples of patients with PDR (n=28), diabetic patients without PDR (n=24), and patients without diabetes with a macular hole (n=10) or macular pucker (n=16), and were related to clinical data, including degree of intra-ocular neovascularization and fibrosis.
Results. TIMP-1, CTGF and VEGF levels, but not activated TGF-β2 levels, were significantly increased in the vitreous of diabetic patients, with the highest levels in PDR patients. CTGF and the CTGF/VEGF ratio were the strongest predictors of degree of fibrosis. However, activated TGF-β2 correlated with TIMP-1 levels in diabetic patients with or without PDR, whereas TIMP-1 levels were associated with degree of neovascularization, like VEGF levels.
Conclusions. We confirm our previous findings that retinal fibrosis in PDR patients is significantly associated with vitreous CTGF levels and the CTGF/VEGF ratio. In contrast, vitreous levels of TIMP-1, associated with levels of activated TGF-β2, were linked with active neovascularization in diabetes, and not with fibrosis. Thus, TGF-β2 and TIMP-1 appear to have a role in the angiogenic phase rather than in the fibrotic phase of PDR.

EFFECT OF FENOFIBRATE ON RETINAL NEURODEGENERATION IN DB/DB MICE

L. Corraliza[1,2], P. Bogdanov[1,2], A. R. Rodrigues-Carvalho[3], J. Garcia-Arumí[3], C. Hernández[1,2], R. Simó[1,2]
[1]Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), ISCIII, Spain; [2]Diabetes and Metabolism Research Unit, Vall d'Hebron Research Institute. Universitat Autònoma de Barcelona, Spain; [3]Ophthalmologic Research Laboratory, Vall d'Hebron Research Institute. Universitat Autònoma de Barcelona, Spain

Introduction. Experimental case-control study.
Purpose. There is now consistent evidence from two major trials, the FIELD and the ACCORD-Eye, that fenofibrate arrests the progression of diabetic retinopathy. Nevertheless, the mechanisms by which fenofibrate exerts these benefits need to be elucidated. The aim of the study was to evaluate the potential effect of fenofibric acid (the active metabolite of fenofibrate) in preventing retinal neurodegeneration in an experimental model of diabetic retinopathy (the db/db mouse).
Methods. A total of 24 diabetic mouse (db/db) (8weeks) were randomly assigned to daily oral treatment (by gavage) with fenofibric acid (100 mg/Kg/day) (n=12) or vehicle (n=12) for one week. We measured the body weight, triglycerides and glucose levels. Retinal neurodegeneration was investigated by measuring glial activation and apoptosis. In addition functional abnormalities were evaluated by electroretinography (ERG) before and after treatment. Glial activation was assessed by immunohistochemistry and western blotting against GFAP (Glial fibrillar acidic protein). Apoptosis was quantified using the TUNEL method. Statistical analysis: Comparisons of quantitative variables between groups were performed using the unpaired Student *t*-test and the Mann-Whitney *U* test.
Results. As expected, lower levels of triglycerides were detected in db/db treated with fenofibric acid in comparison with db/db treated with vehicle ($p=0.02$). No differences in blood glucose and body weight were detected between the groups. Treatment with fenofibric acid significantly prevented the glial activation ($p<0.001$) and the neuronal apoptosis ($p<0.05$). Moreover, db/db mice treated with fenofibric acid showed a dramatic improvement in ERG parameters (b-wave implicit time and oscillatory potentials amplitude).
Conclusions. Fenofibric acid prevents retinal neurodegeneration induced by diabetes. Our results suggest that neuroprotection could be added to the non-lipidic mechanism by which fenofibrate exerts its beneficial actions in diabetic retinopathy.

BENEFICIAL EFFECTS OF SOMATOSTATIN IN CELLULAR VIABILITY OF RCG5 CELLS AND RETINAL EXPLANTS UNDER DIABETIC CONDITIONS: POSSIBLE ROLE OF PROTEIN TYROSINE PHOSPHATASE 1B

A. I. Arroba[1,2], D. J. Burks[2,3], C. Hernandez-Pascual[2,4], R. Simó[2,4], A. M. Valverde[1,2]
[1]Institute of Biomedicine Alberto Sols (CSIC/UAM), Madrid, Spain; [2]Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), ISCIII, Spain; [3]Research Center Príncipe Felipe, Valencia, Spain; [4]Diabetes and Metabolism Research Unit. Vall d'Hebron Research Institute. Universitat Autónoma de Barcelona, Spain

Introduction. This is an experimental study.
Purpose. We aimed to evaluate the effect of somatostatin (SS) on cellular viability of retinal ganglion cells and retinal explants. In addition,

we investigated the possible effects of SS by modulating protein tyrosine phosphatase 1B (PTP1B).
Methods. RCG5 cells were cultured under normoxia or hypoxia (1% oxygen) either with normal (5 mM) or high (55 mM) glucose in the absence or presence of SS (10-6 M). Cellular viability was analyzed by crystal violet staining along 72 h of culture. Retinas were isolated from 3 month-old male mice (C57/BL6 × 129 sv) and cultured in R16 medium in the conditions described above. Total RNA was isolated and PTP1B and VEGF mRNA levels were determined by quantitative PCR.
Results. Under normoxic conditions, RGC5 cells proliferate along 72 h of culture regardless the presence of normal or high glucose in the culture medium. In these experimental conditions, SS did not affect the proliferation rate. However, cellular viability of RCG5 cells cultured in hypoxic conditions under normal glucose significantly decreased at 48 h and was maintained at similar low levels up to 72 h. The presence of high glucose further decreased cellular viability at 72 h. SS was able to recover cellular viability of RCG5 cells cultured under hypoxia and hyperglycemia.
Due to the involvement of PTP1B in cell proliferation-mediated signaling, we determined PTP1B mRNA levels in retinal explants cultured under conditions of diabetic milieu. PTP1B mRNA levels significantly increased at 72 h of culture of retinal explants in high glucose and both normoxia or hypoxia. The presence of SS was able to decrease PTP1B mRNA levels. To further assess our experimental conditions in retinal explants, we analyzed VEGF mRNA levels. Hypoxia and hyperglycemia induced VEGF mRNA at 24 h, these effects being significantly inhibited in the presence of SS.
Conclusions. Our results indicate that SS is involved the maintenance of viability of RCG5 cells in conditions of diabetic milieu. Moreover, data in retinal explants suggest that modulation of PTP1B might be a potential molecular mechanism for this effect.

THE NEUROPROTECTIVE DRUGS SOMATOSTATIN AND BRIMONIDINE DO NOT SHOW ADVERSE EFFECTS ON HUMAN RETINAL PERICYTES

T. Lopatina, E. Beltramo, M. Porta
Departrment of Medical Sciences, University of Turin, Italy

Introduction. Diabetic retinopathy (DR) is generally considered a microcirculatory disease. However, there is growing evidence that retinal neurodegeneration may also occur early in its pathogenesis. While retinal vascular and metabolic damage have been widely addressed by several studies in the literature, neuronal apoptosis caused by hypoxia and diabetic dysmetabolism, an essential target for pharmacological studies, needs further investigations.
Purpose. The aim of this study was to verify preliminarily the effects of the neuroprotective drugs somatostatin and briminodine on human retinal pericytes, which are the earliest vascular cells affected by diabetic retinopathy, in order to exclude possible negative effects of these drugs on retinal microvessels.
Methods. Human retinal pericytes were exposed intermittently at 48 hr intervals in high (28 mM) / normal (5.6 mM) glucose for 8 days (intHG), with or without the addition of 10e-8, 10e-7, 10e-6 M somatostatin or 10e-9, 10e-8, 10e-7 M brimonidine. Control cells were cultured in stable physiological (NG) or high glucose (HG), with or without somatostatin or brimonidine at the same concentrations as above. Cell proliferation was assessed by BrdU incorporation, apoptosis (DNA fragmentation) by ELISA, while a fluorescent/chemioluminescent assay was used to measure viability, cytotoxicity and apoptosis (caspase 3/7 activity) in the same wells. Cytotoxicity and apoptosis data were normalized by viability (rate of death / rate of apoptosis).
Results. Apoptosis increased and proliferation decreased in human retinal pericytes exposed to intermittent glucose concentrations only, accordingly to previous data from our laboratory. Somatostatin and brimonidine, at all concentrations tested, did not show any significant effect, either positive or negative.
Conclusions. We can conclude that the neuroprotective drugs somatostatin and brimonidine do not exert adverse effects on retinal pericytes. Further studies will be addressed to verify the effects of the two drugs on retinal ganglion cells/pericytes and endothelium/pericytes co-cultures in high glucose and hypoxic conditions, in order to mimic the diabetic retinal milieu.

NOVEL NONPEPTIDE SUPEROXIDE DISMUTASE (SOD) MIMETICS ATTENUATE HYDROGEN PEROXIDE-INDUCED DAMAGE TO RETINAL GANGLION CELLS AND HUMAN RETINAL PERICYTES

A. Oknianska[1,2,3], U. Popowska[2], M. Gorska[2], N. Knap[2], A. Daca[3], S. P. Harding[1], M. Wozniak[2]
[1]Department of Eye and Vision Science, Faculty of Health and Life Sciences, University of Liverpool, Liverpool, UK; [2]Department of Medical Chemistry, Medical University of Gdansk, Gdansk, Poland; [3]Department of Pathophysiology, Medical University of Gdansk, Gdansk, Poland

Introduction. Diabetic retinopathy (DR) is characterised by microvascular damage and neurodegeneration of the retina. Retinal ganglion cells (RGC) and capillary pericytes (HRP) are the first cells damaged in DR, however the mechanisms responsible for the apoptotic cell death or survival remain unclear. Oxidative stress contributes to RGC death and HRP loss in the course of diabetic retinopathy, and the administration of low molecular SOD mimetics can possibly enhance oxidative stress defence mechanisms. Here, we examined the cytoptotective efficacy of novel SOD mimetics, being acyl esters of TEMPOL:butyryl-TEMPOL (tempol C-4),octanoyl-TEMPOL (tempol C-8),palmitoyl-TEMPOL (tempol C-16) on H2O2-treated RGC-5 cells and HRP cells cultured in DMEM supplemented with glucose at physiological concentration of 5.5 mM (low glucose=LG) or pathological concentration of 25 mM (high glucose=HG).
Purpose. The aim of this study is to determine whether RGC-5 and HRP are more susceptible to H2O2-induced injury under hyperglycaemic conditions, and to investigate whether new nonpeptide SOD mimetics could protect these cells against H2O2 -induced damage.
Methods. RGC-5 (3 × 105 cells/well) and HRP (7 × 104 cells/well) were plated in six-well culture dishes in DMEM supplemented with LG or HG for 24 hrs, and after pretreatment with TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy) or TEMPOL esters for 1 hr, H2O2 was added to the cultures for 24 hrs. The cells were washed, harvested by trypsinisation, stained with PI or Annexin V-FITC and PI (FITC Annexin V Apoptosis Detection Kit # 556547, BD Pharminogen) and analysed by flow cytometry (FACScan system, Becton Dickinson, San Jose, CA).
Results. Flow cytometry showed a significantly higher rate of apoptosis in HRP cells exposed to HG and 100 μM H2O2 compared to control cells cultured in HG DMEM (92.8±3.2% versus 23.6±9%, $p<0.0001$). The percentage of PI stained RGC-5 cells was significantly higher in cells exposed to HG and 100 μM H2O2 than in cells cultured in DMEM supplemented with LG and treated with 100 μM H2O2 (93.7±6% versus 10.2±4.6%, $p<0.0001$). Pretreatment of RGC-5 cells with 20 μM tempol C8 or tempol C4 reduced H2O2-induced cell apoptosis from 76.7% (HG + H2O2 treated cells) to 15.6% or 42.7%, respectively.
Conclusions. RGC-5 and HRP cells cultured in DMEM supplemented with HG proved more susceptible to H2O2-induced damage than the cells cultured in DMEM supplemented with LG. Tempol C-8, and to a lesser extent tempol C-4, exhibited a remarkable cytoprotective

effect on H2O2-treated RGC-5 cells, and showed a tendency towards improving cell survival of H2O2-treated HRP cells.

A SHIFT IN THE BALANCE OF VEGF AND CTGF BY BEVACIZUMAB CAUSES THE ANGIO-FIBROTIC SWITCH IN PROLIFERATIVE DIABETIC RETINOPATHY

M. Dallinga[1], I. Klaassen[1], R. J. Van Geest[1,2], S. Y. Lesnik-Oberstein[2], R. Goldschmeding[3], C. J. F. Van Noorden[1], R. O. Schlingemann[1,2,4]

[1]Ocular Angiogenesis Group, Departments of Ophthalmology and Cell Biology and Histology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; [2]Department of Ophthalmology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; [3]Department of Pathology, University Medical Center Utrecht, Utrecht, The Netherlands; [4]Department of Clinical and Molecular Ophthalmogenetics, Netherlands Institute for Neuroscience, Royal Netherlands Academy of Arts and Science (KNAW), Amsterdam, The Netherlands

Introduction. Vitreous samples of a prospective cohort of patients with PDR, and patients with PDR that received bevacizumab were analysed and correlated to the clinical degree of neovascularisation and fibrosis.

Purpose. In proliferative diabetic retinopathy (PDR), vascular endothelial growth factor (VEGF) and connective tissue growth factor (CTGF) may cause blindness by neovascularisation followed by fibrosis of the retina. It has previously been shown that a shift in the balance between levels of CTGF and VEGF in the eye is associated with this angiofibrotic switch. This study investigated whether anti-VEGF agents induce accelerated fibrosis in patients with PDR, as predicted by this model.

Methods. CTGF and VEGF levels were measured by ELISA in 52 vitreous samples of PDR patients, of which 24 patients had received intravitreal bevacizumab 1 week to 3 months before vitrectomy, and were correlated with the degree of vitreoretinal fibrosis as determined clinically and intra-operatively.

Results. CTGF correlated positively, and VEGF correlated negatively with the degree of fibrosis. The CTGF/VEGF ratio was the strongest predictor of fibrosis. Clinically, increased fibrosis was observed after intravitreal bevacizumab.

Conclusions. These results confirm that the CTGF/VEGF ratio is a strong predictor of vitreoretinal fibrosis in PDR, and show that intravitreal anti-VEGF treatment causes increased fibrosis in PDR patients. These findings provide strong support for the model that the balance of CTGF and VEGF determines the angiofibrotic switch, and identify CTGF as a possible therapeutic target in the clinical management of PDR.

ESTIMATING THE INTERVAL PRECEDING DIAGNOSIS OF TYPE 2 DIABETES FROM THE TIME-COURSE OF RETINOPATHY PREVALENCE

M. Porta[1], G. Curletto[1], E. Coppo[1], A. Raviolo[1], E. Sitia[1], S. Albani[1], P. Dalmasso[2]

[1]Department of Medical Sciences, University of Turin, Italy; [2]Department of Public Health and Paediatric Sciences, University of Turin, Italy

Introduction. Type 2 diabetes (T2DM) has a slow onset and remains undiagnosed for years. This interval is estimated longer than 10 years, by extrapolating the correlation between known diabetes duration and prevalence of diabetic retinopathy (DR). However, those calculations were based upon weak assumptions: 1) that such correlation is linear, 2) any degree of DR was considered, including mild lesions observed in 10% of non diabetic individuals, 3) uncertain definition of T2DM, 4) time from onset of diabetes to appearance of DR arbitrarily set at 5 years.

Purpose. To estimate the interval preceding diagnosis of T2DM after correcting the above assumptions

Methods. 12,074 patients (35,545 screenings) were stratified into Younger-Onset (YO) if age at onset was <30 or Older-Onset (OO, ≥30) and currently on insulin treatment (IT) or not (NIT). DR was classified as mild or more severe (AnyDR) and moderate or more severe (ModDR). The best-fits between known duration of diabetes and prevalence of DR were calculated by the coefficient of determination (R2) and Akaike's information criterion (AIC). Time preceding diagnosis of T2DM was estimated by adding the negative intercept on the horizontal axis of the best-fitting correlation line between known duration of OO-NIT and prevalence of DR to the positive intercept of the best-fitting correlation between duration of YO-IT and appearance of DR.

Results. There were 7,298 OO-NIT (equivalent to T2DM), 1,719 with AnyDR and 685 with ModDR, and 1,725 YO-IT (equivalent to T1DM), 756 with AnyDR and 385 with ModDR. In the OO-NIT the best-fits were a quadratic model suggesting appearance of AnyDR 3.89 years before diagnosis of T2DM and a linear model with ModDR appearing 2.66 years before diagnosis. The onset of AnyDR following onset of YO-IT could not be timed, whereas a quadratic model suggested appearance of ModDR 3.29 years after its onset. The resulting estimate was (2.66+3.29) 5.95 years preceding T2DM diagnosis.

Conclusions. Use of appropriate fitting models and stratification by treatment and DR severity lowers the estimate of unknown duration before diagnosis of T2DM to about 6 years, which is more realistic than previous estimates.

BASELINE PREDICTORS OF 3-YEAR RESPONSES TO RANIBIZUMAB AND LASER PHOTOCOAGULATION THERAPY IN PATIENTS WITH VISUAL IMPAIRMENT DUE TO DIABETIC MACULAR EDEMA (DME)

V. Chong[1,2], P. Mitchell,[2]

[1]Oxford Eye Hospital, Oxford, UK; [2]Eye Clinic (B4A), Westmead Hospital, Westmead, NSW, Australia

Introduction. RESTORE was a randomised, phase III study in DME that compared 12-month clinical outcomes with laser photocoagulation (LPC), ranibizumab 0.5 mg, and ranibizumab 0.5 mg plus LP.

Purpose. DME is a vision-threatening consequence of diabetes. Accurate means of predicting treatment responses are needed. We used data from RESTORE to identify baseline factors predicting long-term visual outcomes.

Methods. Patients who completed RESTORE were eligible for enrollment into a 24-month extension. Patients could receive ranibizumab as-needed according to pre-specified criteria similar to the EU label. At Month 12, patients remaining on the study were eligible for ranibizumab 0.5 mg as needed. Change from baseline in best-corrected visual acuity (BCVA) at 36 months was analysed using multivariate linear regression adjusting for baseline characteristics (separately for each group: those who received LPC only, and those who received ranibizumab with or without LPC [RBZ]).

Results. BCVA gains with RBZ were maintained with declining injection frequency. In patients treated with RBZ or LPC only in Year 1, (n=166 and n=74), the baseline characteristic most strongly associated with change in BCVA was baseline VA (inverse; p=0.0003 and p<0.0001). RBZ patients who were older, or who had diabetes or DME for longer, had poorer VA outcomes (p=0.045, p=0.013, and p=0.045). In LPC patients, VA changes according to most of these baseline characteristics were numerically similar to those in RBZ patients but were not statisti-

cally correlated, except for duration of DME, which was lower in LPC patients. In LPC only patients, baseline central retinal thickness was inversely associated with change in BCVA (p=0.0063), which was not evident for RBZ patients and not significant under univariate analysis.
Conclusions. This exploratory analysis suggests that baseline BCVA is a strong predictor of BCVA changes over 36 months. Patients with poorer VA achieved greater gains than those with better vision. Longer duration of DME is associated with poorer outcomes, suggesting the need for prompt therapy. Variations in size and strength of associations per initial treatment are possibly due to different anatomical and functional responses to ranibizumab and LPC.

PATIENT-REPORTED VISUAL FUNCTION IN DIABETIC MACULAR EDEMA: 36-MONTH RESULTS FROM THE RESTORE EXTENSION STUDY

P. Massin[1], P. Mitchell[2], J. Petrillo[3], C. D. Coon[4], A. Ferreira[5], N. Bressler[6]
[1]Service d'Ophtalmologie, Hôpital Lariboisière, Paris, France; [2]Dept of Ophthalmology, University of Sydney, Sydney, Australia; [3]Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA; [4]RTI Health Solutions, Research Triangle Park, NC, USA; [5]Novartis Pharma AG, Basel, Switzerland; [6]Retina Division, Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Introduction. A 12-month randomized controlled trial, with additional follow-up of 24 months.
Purpose. To explore changes in the National Eye Institute Visual Function Questionnaire-25 (NEI VFQ-25) scores among RESTORE Extension patients.
Methods. Patients with vision impairment due to diabetic macular edema (DME) were randomized to ranibizumab+sham laser (R), ranibizumab+laser (R+L), or sham injection+laser (L). From the 12-month visit onward (RESTORE Extension entry), all participants could receive ranibizumab as needed depending on whether visual acuity was stable. Change in NEI VFQ-25 score was assessed using the last observation carried forward approach.
Results. Of the 83/116 (72%), 83/118 (70%), and 74/111 (67%) patients in the R, R+L, and L groups, respectively, enrolled in RESTORE who entered the Extension study, 73/83 (88%), 72/83 (87%), and 63/74 (85%) completed 24 additional months of follow-up, including 62/83 (75%), 67/83 (81%), and 59/74 (80%) who received ranibizumab during the Extension. From 0 to 36 months, the least square mean (SE) NEI VFQ-25 composite score increased by 4.1 (1.7), 4.0 (1.7) and 4.1 (1.8) for the originally assigned R, R+L, and L groups, including a change from 12 to 36 months of –1.8 (1.5), –1.0 (1.4) and +1.6 (1.5) respectively. Similar trends were noted for near activities, mental health, distance activities, and general vision subscales. Improvement at 36 months in the mean (SE) composite score was seen across groups initially treated with ranibizumab when stratified by whether the treated eye was the better- or worse-seeing eye at baseline: 8.7 (3.3) and 3.4 (2.0), respectively for R and 9.2 (3.1) and 3.5 (2.1) respectively for R+L, compared with 5.8 (4.3) and 3.1(2.3) respectively for L.
Conclusions. This exploratory analysis suggests that improvements in visual functioning at 12 months among ranibizumab-treated patients are sustained through 36 months. Patients receiving initial laser treatment also had improvements in composite score, general vision, mental health, and near activities at 36 months.

AN EVALUATION OF OPHTHALMIC PHOTOGRAPHIC DIABETIC REVIEW (OPDR) WITH TWO FIELDS DIGITAL PHOTOGRAPHY FOR DIABETIC MACULOPATHY (M1) AND 4 YEAR FOLLOW UP

H. Fellows, M. Shah, A. Hamid, L. Quant, M. Clarke, P. M. Dodson
Heart of England Foundation Trust, Birmingham, UK

Introduction. Retrospective Analysis.
Purpose. The NHS Diabetic Eye Screening Programme states that from April 2013 all programmes should provide a virtual photographic clinic (OPDR) in order to reduce the number of unnecessary referrals into the Hospital Eye Service (HES). This study aimed to evaluate the OPDR pathway as a means to manage patients with diabetic maculopathy (M1) and to investigate whether patients develop M1 in the fellow eye.
Methods. A 4 year retrospective analysis of 455 patients within the Birmingham, Solihull and Black Country Screening Programme who presented with M1 at Diabetic Eye Screening in 2008 and were subsequently placed on OPDR at Heartlands Hospital.
Results. The mean age of patients who presented with M1 was 60 years (18-87). At the end of the 4 year follow up 280 (61.5%) patients were put back to annual recall (AR), 141 (30.9%) were referred into HES, 1 (0.2%) patient is still being monitored in OPDR and 33 (7.25%) patients either did not attend (DNA) their appointment(s) or are deceased. The mean number of OPDR visits for patients who were deemed safe for AR was 1.40 and the mean for number of visits for patients accepted into HES was 1.71. Of the 455 patients, 89 (19.5%) developed M1 in the fellow eye.
Conclusions. OPDR is an effective tool in managing patients with maculopathy and reduces unnecessary referrals to HES, saving on cost and resources to the NHS.

CLINICAL AND DEMOGRAPHIC FEATURES OF PATIENTS WITH DIABETIC MACULAR OEDEMA

F. Afshar, D. Baumgardt, C. Bailey
Bristol Eye Hospital, UK

Introduction. A retrospective review.
Purpose. Diabetic macular oedema is an important cause of visual loss, and the number of patients with diabetes and subsequently diabetic macular oedema is expected to increase. Recently the National Institute of Health and Clinical Excellence have approved the use of Ranibizumab for the treatment of diabetic macular oedema (DMO) greater than 400 microns. We reviewed our patients with diabetes to assess the number which would be eligible for this treatment and evaluated their clinical characteristics.
Methods. The electronic patient records for patients with DMO were reviewed using the Medisoft software program over the past 5 years. DMO was measured using a Topcon OCT system.
Results. There are 31000 patients in our diabetic screening service of which 3600 are reviewed in the hospital eye service. In our local population there were 600 patients diagnosed with DMO. The mean central subfield thickness in this group was 346 microns. 25% of these patients had central subfield thickness greater than 400 microns. Further analysis of this subgroup revealed that 44% were female and 56% male. The mean age was 64 years and 88% had type 2 diabetes. The mean visual acuity was 6/36 snellen equivalent and 74% had visual acuity <6/12. 31% of had active proliferative diabetic retinopathy and 50% had been treated with pan retinal laser previously. 76% had received previous macular laser.
Conclusions. The data provides important and accurate clinical and demographic data, over a 5 year period, for a population with DMO, and highlights the degree of visual morbidity in this population.

Poster Sessions

DIABETIC RETINOPATHY SCREENING AT A UK DISTRICT GENERAL HOSPITAL

F. Blain, S. Shafquat
Russells Hall Hospital, West Midlands, UK

Introduction. Retrospective audit of all patients referred from United Kingdom (UK) national diabetic retinopathy screening programme to a district hospital over a period of 3 months.
Purpose. Diabetic Retinopathy (DR) is the most common cause of visual loss in the working age population in the UK. In 2006, the English National Screening Programme for Diabetic Retinopathy – now NHS Diabetic Eye Screening Programme, was set up with the aim to reduce visual impairment due to diabetic eye disease.
This audit aims to evaluate how many patients are referred to tertiary grading and how many of these are accepted. The second outcome was to assess whether those accepted are seen and treated within the recommended time frame (Timeline analysis). Furthermore, we determined how many patients, once accepted from screening, stayed under the care of the Hospital Eye Service (HES) and/or had treatments for other conditions.
Methods. Retrospective audit of all patients referred from Diabetic Retinopathy Screening to a UK district general hospital over a period of 3 months in 2011. Data were collected from original tertiary grading, departmental database and case notes. Data collection and basic statistical analysis were undertaken in Excel.
Results. 58% of 384 submissions were accepted from tertiary grading. 82% of referrals were seen within the recommended time frame, and 85% of lasers were carried out in time. 28% of patients could be discharged back to community screening, 29% remained under the HES for monitoring of DR and 15% for other conditions. 9% of patients required surgery, out of which 86% was cataract surgery, 9% ranibizumab injections and 5% vitrectomy. 4% of patients accepted from screening did not attend or appointments were rescheduled, and 7% of patients were already under HES care.
Conclusions. Despite the high demand and volume, the majority of patients are seen and treated within the recommended time frames. In cases the targets were not met, over 60% of the time this was due to patient non-attendance or rescheduling, necessitating more patient-led appointment provision. It was also suggested that Outpatient Diabetic Review be started in community to reduce workload in secondary care.

IS IT SAFE TO INCREASE DIABETIC RETINOPATHY SCREENING INTERVAL IN PATIENTS WITH NO/BACKGROUND DIABETIC RETINOPATHY?

H. Chambers[1], S. B. Balu[1], H. W. Wharton[1], P. M. D. Dodson[1,2], J. M. G. Gibson[1,2]
[1]Departments of Diabetes and Ophthalmology, Birmingham Heartlands Hospital, UK; [2]School of Health and Life Sciences, Aston University, Birmingham, UK

Introduction. A 4 year retrospective follow up of 996 patients who presented with no DR and 500 with background DR at baseline digital DR screening in 2006.
Purpose. To evaluate the safety of increasing screening intervals in patients with no diabetic retinopathy (DR) or with background DR.
Methods. A 4 year retrospective follow up of 996 patients who presented with no DR and 500 with background DR at baseline digital DR screening in 2006.
Results. Background DR Group: Of the 500 subjects that had background DR in 2006, 231 were referred for DR, with an average DR routine referral rate of 12% (46 subjects) per year.
No DR Group. Of the 996 patients who had no DR at baseline, 51 were referred over the 4 years for sight threatening DR (STDR), of these 45 patients have definite STDR confirmed by ophthalmological examination. 78% of these had type 2 diabetes and mean age at referral was 60 years (25-87). Mean diabetes duration was 10.7 years (3-32), with a mean HbA1c of 7.8% (5.7-11.3%).
Eight patients (0.9%) were referred in the first year, 9 (0.9%) in the second year, 19 (1.9%) in the third year and 15 (1.5%) in the fourth year. 86% of referrals were for maculopathy, and all had observable retinopathy and none required ophthalmology clinic assessment or laser treatment.
If biannual screening was adopted for patients with no DR at baseline, allowing for patients who subsequently develop background DR and would then revert to annual screening, a total of 7 (0.7%) patients would not have been appropriately referred for STDR and would have waited a further year for identification. None of the 51 referrals across the 4 years required laser treatment apart from just one patient who developed PDR in year 4 (2010) and had background since 2007.
Conclusions. It could be recommended that it is safe to screen patients with no DR biannually due to the low risk of developing STDR. However, patients who present with background DR should continue to be screened annually as there is a significant proportion developing STDR and would not be identified at an appropriate screening interval.

EPIRETINAL MEMBRANE IN DIABETIC RETINOPATHY SCREENING

N. Bilkhu[1], A. D. Wright[1], A. Farnsworth[1], S. Osei-Amoako[1], P. M. Dodson[1]
[1]Birmingham, Black Country and Solihull Diabetic Retinopathy Screening Programme, Birmingham, UK; [2]Birmingham Heartlands Hospital, Bordesley Green East, Birmingham, UK

Introduction. Retrospective analysis.
Purpose. To determine how often epiretinal memebrane (ERM) occurs in a diabetic retinopathy (DR) screening programme and the effects on grading.
Methods. Patients within our screening programme in 2008 (68,637 patients) with mention of ERM or cellophane maculopathy were reviewed. Screening involved two digital photographic 45° images, one macula and one disc centred, through dilated pupils. 422 patients had good quality photos and a definite ERM. ERM was considered idiopathic in the absence of significant history and other ocular pathology. The value of two views was assessed by one senior and one primary grader in a subset of 25 patients.
Results. 422 patients (457 eyes) with ERM (0.61% of total screened). 213 male, 209 female; mean age 71.6±9.5 yrs. ERM was unilateral in 387 (91.7%) (R 181 [46.8%]; L 206 [53.2%]) and bilateral in 35 (8.3%). ERM was idiopathic in 397 (94.1%) and secondary in 25 patients

(5.9%). Visual acuity with ERM was 6/6 or better in 161 (35.2%), 6/9 in 172 (37.7%) and ≥6/12 in 124 (27.1%) eyes. There was no other cause evident for VA ≥6/12 except ERM in 84 (67.8%) eyes. All eyes but one (456 eyes) had been given a DR grade (R0M0 251, R1M1 123, R2M0 5, R2M1 4, R3M0 11, R3M1 6 and ungradable 1). 251 eyes (55.1%) posed grading difficulties. Both senior and primary graders considered the two views were essential in almost all of the subset.
Conclusions. ERM occurred in 0.61% of patients in our screening programme. Patients were elderly and the ERM was idiopathic in 94%. Grading of DR was difficult in 55%, but two views enabled adequate assessment of the great majority of patients with ERM.

IS ARBITRATION OF ALL ABNORMAL GRADES VALUABLE IN RETINOPATHY GRADING QUALITY ASSURANCE?

L. Quant, A. P. Mills, A. Hamid, H. Wharton, M. Clarke, P. M. Dodson
Birmingham, Solihull and Black Country Diabetic Eye Screening Programme, Retinopathy Screening Centre, Birmingham Heartlands Hospital, Birmingham, UK

Introduction. A retrospective analysis of data from arbitration grading within the Birmingham, Solihull and Black Country Diabetic Eye Screening Programme.
Purpose. Our local diabetic eye screening programme assesses image sets using the English National Grading Protocol. All sets (primary) graded abnormal are quality assurance (secondary) graded 'blind'. Disagreements are arbitrated. The patient is then either returned to annual screening or referred to an ophthalmology gatekeeper. In October 2010 we stopped arbitrating differences between normal (R0 M0) and background diabetic retinopathy (R1 M0). The purpose of this audit is to demonstrate this arbitration change was safe and cost efficient.
Methods. 12 months of data from either side of the arbitration change were gathered from our screening database using Structured Query Language (SQL) for comparison. For the year subsequent to the arbitration change SQL was used to show how many patients would have been arbitration graded had our Optomize screening software still been configured to do so.
Results. In the year prior to the change (01/11/2009 to 31/10/2010) 82,697 patients were primary graded. 19,902/82,697 were R1 M0 worst eye - annual recall. 4,277/19,902 arbitrated on an R1/R0 difference. 71/19,902 were referable at arbitration. 12/19,902 were accepted into ophthalmology by the gatekeeper (all routine). 2/12 were accepted for diabetic retinopathy. In the subsequent year (01/11/2010 to 31/10/2011) 84,698 patients were primary graded. 21,049/84,698 were R1 M0 worst eye - annual recall. 6,111/21,049 would have arbitrated if we still arbitrated on R1/R0. By not arbitrating on R1/R0 differences, our total arbitration grades were reduced by 6,111 to 6,031 (50.3%).
Conclusions. Given our living diabetic population of approximately 155,000 a reduction of 6,111 arbitration grades could be scaled up to a reduction of 70,920 grades for an approximate national population of 1.8 million. In the prior year only 2/19,902 (0.01%) patients with R1 worst eye annual recall were accepted into ophthalmology for diabetic retinopathy via arbitration. Both referrals were routine. This demonstrates the overall safety of not arbitrating R1/R0 differences.

EVALUATION OF AUTOMATED IMAGE ANALYSIS SOFTWARE FOR THE DETECTION OF DIABETIC RETINOPATHY TO REDUCE THE OPHTHALMOLOGIST WORKLOAD

E. Soto-Pedre[1], A. Navea[2], J. Morales[2], S. Millan[2], M. C. Hernaez-Ortega[1], P. Perez[2], M. C. Desco[2]
[1]European Innovative Biomedicine Institute (EIBI), Cantabria, Spain; [2]Fundacion Oftalmologica del Mediterraneo (FOM), Valencia, Spain

Introduction. Cross-sectional observational epidemiologic study.
Purpose. To assess the safety of an automated "disease/no disease" grading system for diabetic retinopathy within a systematic screening program.
Methods. A single central field digital image per eye was obtained from consecutive patients attending a regional primary care based diabetic retinopathy screening program in Valencia (Spain). Photographers were trained nurse assistants. The sensitivity and specificity of the automated system (iGrading-Medalytix Ltd- operating as more than one microaneurysm detection for disease presence) grader were determined against a manual grading as gold standard.
Results. 5517 patients were screened at twenty-seven Health Centers of four Health Departments. The median age was 69 years (IQR 16 years) and 56% were male. The software classified 44.5% of the patients as having no retinopathy. Detection of retinopathy was achieved with 94.5% sensitivity (95% CI 92.6-96.5) and 69.1% specificity (95% CI 67.6-70.6).
Conclusions. Automated grading of diabetic retinopathy based on a single field plus one microaneurysm detection for disease presence approach seems to be adequate not only in terms of workload reduction but also in terms of safety.

REVIEW OF CHANGE IN REFERRAL RATES TO HOSPITAL EYE SERVICES FROM A POPULATION BASED DIABETIC RETINOPATHY SCREENING SERVICE DURING THE INITIAL SIX YEARS OF SERVICE DELIVERY

M. Morgan[1,2], M. Murphy[2]
[1]Diabetic Retinopathy Screening Service, St. Conal's Hospital, Letterkenny, County Donegal, Ireland; [2]Department of Public Health, St. Conal's Hospital, Letterkenny, County Donegal, Ireland

Introduction. Retrospective review of referral rates 2006-2011.
Purpose. To look at referral rates from a newly established diabetic retinopathy screening service to guide in planning for service extension to ensure capacity within the hospital eye services for management of referrals.
Methods. Retrospective data collection of referral rates for sight threatening and non sight threatening diabetic retinopathy, non diabetic eye disease and for upgradable images from 2006-2011. The percentages of urgent referrals R3 (proliferative DR) and non-urgent for R2 (severe non-proliferative DR), M1 (maculopathy) were also collected. Currently all people over 12 with a diagnosis of diabetes are screened including those in Ophthalmic care.
Results. The screening cohort increased from 2384 in 2006 to in 7322 in 2012. Referral rates for sight threatening diabetic retinopathy decreased from 15.2% of screened patients in 2006 to 9.8% in 2011, for non-sight threatening DR from 1.2% in 2006 to 0.9% in 2011, for non-diabetic eye disease from 4.8% in 2006 to 2.5% in 2012, referrals for upgradable images from 5.2% in 2006 to 2.6% in 2011. The percentage of patients referred for ungradable images reduced from 5.2% in 2006 to 3.5% in 2007. There had been a service review in mid

2007 following which all patients received mydriatic drops prior to this mydriasis was performed at the discretion of the photographer. The percentage of urgent referrals relative to total referrals reduced from 20.6% in 2007 to 10.1% in 2011.
Conclusions. There has been a gradual reduction in referral rates for diabetic retinopathy, non diabetic eye disease, ungradable images and urgent referrals since the inception of the service. The reduction the numbers of patients referred for ungradable images from 2006-2007 may be explained by the change to screening with mydriasis. Analysis of referral rates from 2013 will yield further information as all patients currently in care of the Hospital Eye Services are to be discharged from screening.

ARE DIABETIC RETINOPATHY VIRTUAL CLINICS SAFE?

O. Giasin, J. Myerscough, C. James, Y. Osoba
Torbay Hospital, South Devon NHS Trust, Devon, UK

Introduction. This was a screening based cohort study.
Purpose. To investigate the effectiveness and safety of virtual clinics in the context of a Diabetic Eye Screening Program (DESP).
Design. Outcomes review one year after initial assessment of a cohort of maculopathy referrals to a virtual clinic (VC).
Methods. Patients undergoing routine screening within the DESP in whom maculopathy was suspected were referred to a virtual clinic instead of the Hospital Eye Service (HES) for initial assessment. The virtual clinic episode comprised of visual acuity testing, retinal photography, macular optical coherence tomography (OCT) and remote review of the images by a consultant ophthalmologist. Patients were divided into four subgroups for follow-up and data collected on their outcomes after 12 months, either in the DESP or the HES. Subgroups were: high risk for immediate referral to HES (group 1), referred to HES after single 6 month follow-up in virtual clinic (group 2), 6 monthly follow-up in virtual clinic (group 3), discharged to DESP after first virtual clinic episode (group 4).
Results. A cohort of 59 patients was studied. Outcomes at 12 months for group 1 (n=12) were: 4 treated for clinically significant macula oedema (CSMO), 2 treated for non diabetes related pathology, 6 followed without treatment. In group 2 (n=25) 1 was treated for CSMO after an abnormal OCT at 6 months in virtual clinic, 4 treated for non diabetes pathology, 20 discharged to DESP. In group 3 (n=8) 1 patient was reviewed in HES with an abnormal OCT in VC, but not treated, 7 discharged to DESP. In group 4 (n=14) a year later, 8 had no maculopathy, 3 were re-referred to the HES but none required treatment, and 3 failed to attend.
Of patients referred from the DESP to the virtual clinic with maculopathy, 22% needed HES treatment or continued assessment while 69% needed virtual clinic assessment +/- short term follow-up only, before being suitable for discharge back to DESP. The 5 patients needing maculopathy treatment were identified in the virtual clinic and were referred and treated in a timely fashion. Of the remaining 51/54 patients who attended for review (3 failed to attend), none had developed maculopathy by 12 months that had been missed during their period of follow-up in the virtual clinic.
Conclusions. Our study suggests that virtual clinics are an effective way of relieving pressure on the HES and are safe.

RELATIONSHIP BETWEEN TIME FROM DIAGNOSIS OF DIABETES TO SCREENING AND GRADING OUTCOME

S. J. Aldington[1], I. M. Stratton[1], M. Histed[2], P. H. Scanlon[1]
[1]Gloucestershire Diabetic Retinopathy Research Group, UK; [2]Gloucestershire Diabetic Eye Screening Programme, UK

Introduction. Data collected from a programme of NHS Diabetic Eye Screening Programme were analysed to examine the proportion with diabetic eye disease at intervals from diagnosis of diabetes.
Purpose. To assess whether those who are screened later are at greater risk of sight threatening diabetic eye disease (STDR).
Methods. Patients were referred from 88 primary care practices to the eye screening programme and invited for screening locally with mobile cameras. Digital retinal images of both eyes were taken after pharmacological dilatation and graded by a quality assured grading team. Data for patients referred to the eye screening programme were extracted from the primary care databases with semi-automated data collection algorithms supplemented by validation processes.
Results. Data were available for 8183 patients newly diagnosed between 2005 and 2007. Only 163 with Type 1 diabetes were available and were insufficient for analysis. Data were available for 8020 with newly diagnosed Type 2 diabetes. Of these 3569 were screened within 6 months, 2361 were screened between 6 and 11 months, 1058 between 12 and 17 months, 366 between 18 and 23 months, 428 between 24 and 35 months, 238 at 3 years or more after diagnosis. There were 5416 (67.5%) graded with no retinopathy, 1629 (20.3%) with minimal background retinopathy (microaneurysms only) in one eye, 753 (9.4%) with background DR in both eyes and 222 (2.8%) had STDR. There was a significant trend (p=0.0004) relating time from diagnosis to screening with worsening DR. Of those screened within 6 months of diagnosis 2.3% had STDR. In those screened 3 years or more after diagnosis 4.2% had STDR.
Conclusions. Whilst it is unclear whether those who were screened later had more severe DR at diagnosis these data show that the rate of detection of STDR is elevated in those who were not screened promptly after diagnosis of Type 2 diabetes. Hence, in a systematic screening programme, resources should be put towards obtaining images in these people as early as possible so that STDR is identified.

ACCURACY OF GLAUCOMA SUSPECT REFERRALS FROM DIABETIC RETINOPATHY SCREENING

T. Peto, K. Balaskas, R. Puertas
NIHR Biomedical Research Centre, at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London, UK

Introduction. Prospective, institutional audit.
Purpose. Tower Hamlets PCT Diabetic Retinopathy Screening Service has an active register of 12879 patients with diabetes. Approximately 733 (based on Apr 10 to Mar 11) of patients are referred yearly to two hospitals, Moorfields Eye Hospital and the Royal London Hospital. On average, about 5% of the referrals are to Glaucoma Clinics. These referrals are based on suspicious discs found on retinal imaging (including cup/disc ratio/haemorrhage on the disc, notching). In the screening process however, patients are not examined for intra-ocular pressure (IOP) or Anterior Chamber Depth. The purpose of the present audit project is to determine the accuracy and appropriateness of referrals to glaucoma clinics for suspicious optic discs made by trained graders on the basis of retinal images obtained for diabetic retinopathy screening.
Methods. All referrals to glaucoma clinics for suspicious optic discs made by trained graders in the context of diabetic retinopathy grading during the period between April 2010 and October 2011 were reviewed.

The same images were graded for typical findings of glaucomatous optic neuropathy by a glaucoma specialist. Outcome letters from glaucoma clinics were reviewed for corresponding patients and definitive diagnosis was recorded. Sensitivity and specificity of glaucoma referrals were calculated.

Results. 106 patients were referred as glaucoma suspects from the Tower Hamlets PCT Diabetic Retinopathy Screening Service over the period of the audit. Out of these patients, 21 (19.8%) would not have been referred if the same images had been graded by a glaucoma specialist. As regards definitive diagnosis at the glaucoma clinic, 8 patients did not present to clinic, 68 patients (69.4%) were classified as having glaucoma or a high degree of suspicion for glaucoma and remained under specialist care, whereas 30 patients (30.6%) were found to be normal and were discharged.

Conclusions. On the basis of our findings we conclude that the vast majority of glaucoma referrals by trained graders were appropriate, would be replicated by glaucoma specialists and led to a significant pick-up rate of glaucoma even though they were based on limited information drawn exclusively from retinal images.

NHS DIABETIC EYE SCREENING PROGRAMME ON-LINE TEST AND TRAINING (TAT): PAST, PRESENT AND FUTURE

S. Aldington[1], I. M. Stratton[1], D. J. Taylor[2], P. H. Scanlon[1,2]

[1]Dept of Ophthalmology, Gloucestershire Hospitals NHS Foundation Trust, UK; [2]NHS Diabetic Eye Screening Programme, c/o Victoria Warehouse, Gloucester, UK

Introduction. On-line monthly grading QA and training support facility.

Purpose. The NHS-DESP TAT facility helps local grader performance monitoring and continuous professional development. Test cases now ground-truthed by 100% consensus agreement amongst teams of 3 members of 36 staff of the Grading College, drawn from the highest-performing TAT users.

Methods. Web-browser accessed facility is used by staff from 89 DR screening services in England. Monthly image sets available 24/7. Disease-weighted sets of DR cases, each two-field 45o retinal images, presented in years 1, 2 and 3 as 30, 20 and 20 cases (eyes) per month respectively. Until October 2012, Users simply assigned Retinopathy (R) and Maculopathy (M) levels according to national protocols but will, after the current 4-month training period, record lesions for full Feature Based Grading. Basic image manipulation tools have been enhanced and example lesion annotation introduced. User-visible results provide proportional agreement and detected feature-lists against the 'system grade' allocated by the Grading College team consensus and against all local and national peers following each month-end. Completed Test cases move into expanding Training library. Direction of TAT is now overseen by a DESP Steering Group led by a clinical topic expert.

Results. After phased implementation during 2009, 1317 people participated during Year 1. Monthly mean (individual range) agreement scores were lowest month 79% (52-95%) to highest 85% (53-98%). Year 2, 1364 Users completed 1+ set with over 900 regularly participating. Year 2 Results. lowest month 77% (55-93%) to highest 87% (60-100%). During Year 3 (to date) 1304 have completed 1+ set with over 900 regularly participating. Year 3 monthly Results. lowest 84% (58-100%) to highest 90% (48-100%). There were significant differences between the monthly mean test results ($p<0.001$) and between individual graders ($p<0.001$). Grading College members regularly participated and achieved significantly higher results scores than the national average with lowest monthly mean 88% (70-95%) to highest 94% (83-100%) ($p<0.0001$).

Conclusions. The facility is heavily used and can identify consistently high performing staff and those potentially needing additional training. Some test cases and months prove more difficult than others.

THE INFLUENCE RISK COMMUNICATION TOOLS ON DIABETES SELF MANAGEMENT

N. Al-Athamneh[1], J. Sturt[2], I. M. Stratton[1], A. Lindenmeyer[1], P. H. Scanlon[1]

[1]Warwick Medical School, University of Warwick, UK; [2]King's College London, UK

Introduction. Qualitative study using semi-structured interviews.

Purpose. 1. To explore the effect of different ways of communicating risk information about DR to people with diabetes. 2. To explore the possible use of risk communication tools to improve diabetes self management.

Methods. A qualitative study was conducted using semi-structured interviews with a convenience sample of 25 participants. Of these, 20 participants were diagnosed with type 2 diabetes (11 White British and 9 South Asians), and 5 participants who were health care professionals (HCPs): (3 White British and 2 South Asians). HCPs were 2 GPs, an ophthalmologist, and 2 retinal screeners. Participants were purposively recruited from primary and secondary care trusts to fall into 5 groups of 5 participants each. 1) No DR (R0) 2): background DR (R1); 3) preproliferative DR (R2); 4) proliferative DR (R3); 5) HCPs.

Risk communication tools were identified from different clinical settings e.g. cancer screening, modified and presented to the participants.

Results. Providing individualised risk information in a clear and attractive style improved patients' understanding of their own risk and therefore may also improve diabetes self management. The use of visual aids was found to facilitate the presentation of risk for those who could not read, or whose their first language was not English. Participants were able to score themselves as high or low risk, a feature which was welcomed by patients, as they liked the idea of assessing their own risk.

Conclusions. Visual tools may be useful in communicating the risk of DR to people with diabetes. Continuing work will now modify, evaluate, and improve a risk tool to communicate risk information about DR to people with diabetes.

TEAR FLUID PROTEOMICS MULTIMARKERS FOR DIABETIC RETINOPATHY SCREENING

A. Csutak[1,2,4], Zs. Torok[2,6], T. Peto[3], E. Csosz[4], A. M. Molnar[5], J. Tozser[3,7], A. Hajdu[2]

[1]Medical and Health Science Center, Department of Ophthalmology, Debrecen, Hungary; [2]University of Debrecen, Faculty of Informatics, Department of Computer Graphics and Image Processing, Bioinformatics Research Group, Debrecen, Hungary; [3]NIHR Biomedical Research Centre, at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London, UK; [4]University of Debrecen, Medical and Health Science Center, Department of Biochemistry and Molecular Biology, Proteomics Core Facility, Debrecen, Hungary; [5]Centre for Research on Inner City Health, Keenan Research Centre Li Ka Shing Knowledge Institute, St. Michael's Hospital, Toronto, Ontario, Canada; [6]Astrid Research Ltd, Debrecen, Hungary; [7]Inno Tears Ltd, Debrecen, Hungary

Introduction. Pilot study.

Purpose. The aim of the project was to develop a novel method for

diabetic retinopathy screening based on the examination of tear fluid biomarker changes. In order to evaluate the usability of protein biomarkers for pre-screening purposes several different approaches were used, including machine learning algorithms.
Methods. All persons involved in the study had diabetes. Diabetic retinopathy (DR) was diagnosed by capturing 7-field fundus images, evaluated by two independent ophthalmologists. 165 eyes were examined (from 119 patients), 55 were diagnosed healthy and 110 images showed signs of DR. Tear samples were taken from all eyes and state-of-the-art nano-HPLC coupled ESI-MS/MS mass spectrometry protein identification was performed on all samples. Applicability of protein biomarkers was evaluated by six different optimally parameterized machine learning algorithms: Support Vector Machine, Recursive Partitioning, Random Forest, Naïve Bayes, Logistic Regression, K-Nearest Neighbor.
Results. Out of the six investigated machine learning algorithms the result of Recursive Partitioning proved to be the most accurate. The performance of the system realizing the above algorithm reached 74% sensitivity and 48% specificity.
Conclusions. Protein biomarkers selected and classified with machine learning algorithms alone are at present not recommended for screening purposes because of low specificity and sensitivity values. This tool can be potentially used to improve the results of image processing methods as a complementary tool in automatic or semiautomatic systems.

THE ROLE OF VASCULAR ENDOTELIAL GROWTH FACTOR AND RENIN-ANGIOTENZIN SYSTEMS IN THE PATHOGENESIS OF DIABETIC RETINOPATHY

D. Lipatov, J. E. Bautina, M. V. Shestakova, A. G. Kuzmin, T. A. Chistjakov
Endocrinology Research Centre, Ophthalmology Department, Moscow, Russia

Introduction. This is an observational study.
Purpose. To investigate the role of vascular endothelial growth factor (VEGF), angiotensin 2 (AT2) and renin in diabetic macular oedema (DMO) and proliferative stages of diabetic retinopathy (DR) in patients with diabetes mellitus (DM).
Methods. Measurement of concentrations of VEGF, AT2 and renin was conducted on samples of anterior chamber fluid of the eye and plasma from patients with diabetes mellitus (DM) and non-diabetic controls. Eyes were assessed for the presence of DMO, proliferative DR (PDR) and neovascular glaucoma (NVG).
Results. 134 patients were recruited in the group with DM (mean age 64.9 years, diabetes duration 14.7 years; mean glycated haemoglobin (HbA1c) 7.8%) and 30 patients in the control group (mean age 66.1 yrs, mean HbA1c 5.8%). The levels VEGF were directly proportional to the stage of DR. Mean intraocular concentration of VEGF was higher in eyes with proliferative DR (113.7 pg/ml), than with non-proliferative DR (59.9 pg/ml) ($p<0.05$). In the control group, intraocular VEGF was 43.4 pg/ml and its concentrations did not correlate with plasma levels ($p=0.35$). Eyes with NVG had much higher anterior chamber VEGF and AT2 concentrations compared to controls: 1149.9 pg/ml and 66.7 pg/ml respectively. 17 patients (age 47.5 yrs, diabetes duration 16.1 yrs; HbA1c 7.7%) with DMO, PDR or NVG were treated with ranibizumab following a standard protocol. Mean central macular thickness (CMT) at baseline was 632±156 microns and mean visual acuity (VA) 0.7. After completion of a 3-4 month course of ranibizumab there was a reduction in mean CMT of 156 microns with reduction in the number of haemorrhages and new vessels, new vessel diameter and an increase of 1 to 3 lines of VA.
Conclusions. A role for VEGF in the pathogenesis of proliferative stages of DR is shown. The use of anti-VEGF therapy is beneficial in DMO, and for stabilization of proliferative stages of DR.

VEGF LEVELS IN PLASMA IN RELATION TO PLATELET ACTIVATION, GLYCEMIC CONTROL AND MICROVASCULAR COMPLICATIONS IN TYPE I DIABETES MELLITUS

R. Schlingemann[1], C. J. F. van Noorden[1], J. C. M. Meijers[3], W. M. Wiersinga[2]
[1]Ocular Angiogenesis Group, Departments of Ophthalmology and Cell Biology and Histology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; [2]Department of Endocrinology & Metabolism, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; [3]Department of Vascular Medicine and Department of Experimental Vascular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Introduction. Prospective cohort study.
Purpose. Increased levels of vascular endothelial growth factor (VEGF) in human plasma samples have suggested that circulating VEGF is a cause of endothelial dysfunction in diabetes mellitus. However, artificial release of VEGF from platelets as a source of VEGF in plasma samples, as also occurs in serum samples, has not been ruled out in these studies.
Methods. We determined VEGF levels in plasma collected in both citrate and in a medium that inactivates platelets, PECT, in a cross sectional cohort of 21 healthy subjects and 64 patients with type I diabetes. In addition, we evaluated whether VEGF levels in both types of plasma correlated with the presence of diabetes, glycemic control, markers of *in vivo* or *ex vivo* platelet activation and degree of diabetic retinopathy and nephropathy.
Results. VEGF levels were invariably low in PECT plasma of both non-diabetic and diabetic subjects and were unrelated to any other diabetes-related variable studied. In contrast, VEGF levels in citrate plasma were 150% higher in diabetic patients than in controls and correlated with diabetes-related variables. Multiple linear regression analysis showed that levels of PF4, a marker for *ex vivo* platelet activation, and HbA1c were the independent predictors of VEGF levels in citrate plasma. Platelet activation, *in vivo* and *ex vivo*, was similar in diabetic persons and controls.
Conclusions. 1) Like serum, citrate plasma is not suitable for reliable measurements of circulating VEGF. 2) The low levels of VEGF *in vivo*, as represented by measurements in PECT plasma in our study, do not support a role of circulating VEGF in endothelial dysfunction in type I diabetes; 3) Higher levels of VEGF in citrate plasma samples of diabetic persons do not represent the *in vivo* situation, but mainly originate from higher artificial *ex vivo* release from platelets correlating with the degree of glycemic control.

MACULAR FUNCTION: BETTER THAN VISUAL ACUITY FOR ASSESSING DIABETIC MACULOPATHY?

A. Raj[1,2], J. N. Sahni[1,2], C. Campa[1,2], G. Czanner[2,3], R. Hagan[4], S. P. Harding[1,2]
[1]St. Pauls Eye Unit, Royal Liverpool University of Hospital, UK; [2]Department of Eye & Vision Science, University of Liverpool, UK; [3]Department of Biostatistics, University of Liverpool, UK; [4]Department of Medical Physics & Clinical Engineering, Royal Liverpool University of Hospital, UK

Introduction. Single-centre, prospective, cohort study.
Purpose. To determine objective and subjective macular function in

the presence of varying severities of diabetic maculopathy.
Methods. Treatment naïve patients with diabetes mellitus (DM) were recruited prospectively into four groups: i) DM but no retinopathy [n=13]; ii) early diabetic maculopathy but no features of clinically significant macular oedema (CSMO) [n=24]; iii) CSMO [n=30]; iv) ischaemic maculopathy, defined as an enlarged foveal avascular zone (>1000 µm) on fluorescein angiography (FA) with disruption of the capillary network [n=9]; and healthy volunteers without DM [n=21]. All subjects underwent best corrected visual acuity (BCVA), optical coherence tomography, FA, multifocal electroretinography (mfERG), oscillatory potentials (OP), microperimetry (MP) and assessment for systemic risk factors. Power calculations determined a sample size of 30 per group. Statistical analysis was performed on SPSS version 20 using ANOVA; post hoc analysis was performed using the Scheffe and Games-Howell tests.
Results. There was no statistically significant difference in BCVA between the groups of subjects with DM (p>0.10). Mean mfERG central ring amplitude was significantly reduced in subjects with maculopathy without CSMO (58.60nV/o2, p=0.018), with CSMO (49.38nV/o2, p<0.001) and ischaemic maculopathy (20.97nV/o2, p<0.001) as compared to healthy controls (77.08nV/o2). Mean OP sum amplitude was significantly reduced in subjects with CSMO (50.13 µV, p=0.001) and ischaemic maculopathy (33.54 µV, p=0.001) as compared to healthy controls (78.80 µV); there was no significant difference between healthy controls and subjects with early maculopathy. Mean MP central sensitivity was significantly reduced in subjects with early maculopathy (15.18dB, p=0.005), CSMO (14.16dB, p<0.001), and ischaemic maculopathy (8.46dB, p<0.001) as compared to healthy controls (18.08dB). There was no significant difference between healthy controls and subjects without retinopathy for any of the variables.
Conclusions. Neural macular function appears to decrease with increasing severity of maculopathy and in the presence of macular ischaemia. These investigative tools appear to be more sensitive markers of macular function than visual acuity.

RELIGHT- RANIBIZUMAB TREATMENT OF DIABETIC MACULAR OEDEMA (DMO) WITH BIMONTHLY MONITORING AFTER INITIAL TREATMENT

I. Pearce
THE RELIGHT Study Group, St. Pauls Eye Unit, Royal Liverpool University Hospital, Liverpool, UK

Introduction. RELIGHT is a UK, prospective, open-label, multicenter, single-arm study.
Purpose. In patients with visual impairment due to DMO (VI-DMO) treated with ranibizumab 0.5 mg, the RELIGHT study investigates whether extending follow-up to bimonthly following initial monthly follow-up for 6 months, will provide and maintain improvements in vision.
Methods. This is a prospective, open-label, multicenter, single-arm study. It evaluates three monthly initiation doses of ranibizumab 0.5 mg, followed by individualised retreatment. Retreatment criteria are based on reductions in VA of >5 letters and/or OCT ≥225 micrometers with monthly review for three months following initiation, and subsequent bi-monthly review out to 18 months. Laser treatment was permitted after 6 months. Data is presented from the primary end-point at month-12.
Results. Of 139 patients screened, 110 initiated treatment, The primary endpoint was reached for the last patient in October 2012. Preliminary analyses show at baseline, patients had a median age 64 (range 37.5 to 82), median HbA1c 7.6% (range 5.4 to 11.7), and mean BP 139/78 mmHg. Median VA improved from 65 letters (6/15) (range 34 to 84) at baseline to 70 letters (6/12) (range 3 to 85), a median change of 5 letters (range -51 to 27) at 12-months. Median central subfield thickness improved from 452.5 µm to 293 µm, representing a median change of -127.5 µm. A median of seven (range 3-9) injections were given, with 23.1% of patients achieving ≥10 letter gain and 4.6% demonstrating ≥10 letter loss.
Conclusions. This regimen provided VA gains and central subfield thickness improvements consistent with those in the pivotal RESTORE and DRCR.net studies. This was achieved with a reduction in monitoring frequency, similar to that seen in year three of the DRCR.net.

AUTOMATED DETECTION OF LEAKAGE SITES IN FUNDUS FLUORESCEIN ANGIOGRAPHY FOR DIABETIC MACULOPATHY

M. T. Kwong[1,2], Y. Zheng[1,2], D. Parry[2], J. N. Sahni[1,2], A. Raj[1,2], S. P. Harding[1,2]
[1]Department of Eye and Vision Science, University of Liverpool, UK; [2]St. Paul's Eye Unit, Royal Liverpool University Hospital, UK

Introduction. Methodological development study.
Purpose. To describe an automated detection technique for the detection of leakage sites shown on fluorescein angiography (FA) and investigate its feasibility for use in assessment of diabetic maculopathy.
Methods. High-resolution FA sequences were acquired on HRA2 (Heidelberg Engineering, Germany) in patients with diabetic maculopathy. One transit-phase (used as reference for image alignment) and one late-phase image were. The image pair was aligned automatically by commercial software (i2k Retina, DualAlign LLC, Clifton, NY). A low-pass filter was applied to the aligned pair to remove any uneven illumination. A difference image was generated by subtracting the transit from the late-phase image, and then subdivided into superpixels – sets of neighbouring pixels grouped according to their similarity such as texture and intensity. The superpixels were classified into two classes using the k-means clustering method; the class with the higher average pixel intensity was identified as leakage. Two method settings were investigated (method 1: single iteration of k-means; method 2: two subsequent iterations of k-means).The performance of both methods were evaluated by an experienced grader (reference standard) following three heuristic detection categories (good 100% correct; adequate ≥50%; poor <50%).
Results. Images from 22 eyes were analysed with the two proposed methods and evaluated. The Mann-Whitney test shows that method 1 performed significantly better than method 2 (p<0.05). Promising results were achieved using method 1 – one image was graded good, 14/22 (64%) were graded adequate, 7/22 (32%) were graded poor. Results from method 2 – 8/22 (36%) were graded adequate, 14/22 (64).
Conclusions. An automated detection technique using superpixels and single k-means clustering shows promising results for automated detection of leakage regions on FA. This framework has the potential to become a useful tool in the assessment of diabetic maculopathy and its treatment.

SIXTEEN-YEAR INCIDENCE OF DIABETIC RETINOPATHY AND PROLIFERATIVE DIABETIC RETINOPATHY IN A NATIONWIDE COHORT OF YOUNG DANISH TYPE 1 DIABETIC PATIENTS

R. B. Pedersen[1,2], M. L. Rasmussen[1,2], T. Peto[3], J. Grauslund[1,2]
[1]Department of Ophthalmology, Odense University Hospital, DK; [2]Clinical Institute, University of Southern Denmark, DK; [3]NIHR Biomedical Research Centre, Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, UK

Introduction. Prospective cohort-study.
Purpose. The aim of this study was to assess long-term incidence of diabetic retinopathy (DR) and proliferative diabetic retinopathy (PDR) and associated risk factors in a Danish population-based cohort of young type 1 diabetic patients.
Methods. Eighty percent of all Danish type 1 diabetic patients below the age of 18 (n=1033) were examined in 1986-89. In 1995, baseline retinopathy was graded and other risk factors were assessed in 324 patients (31.4% of the original cohort). Of these, 132 (40.7%) were re-examined at follow-up in 2011. At baseline two-field fundus photographs were taken and at follow-up seven-field. All retinal photographs were graded using the ETDRS protocol. In logistic regression, results were correlated to the baseline level of HbA1c, albumin excretion (AER), vibratory threshold, systolic and diastolic blood pressure, BMI, age, gender and diabetes duration.
Results. The mean age and diabetes duration at baseline were 21.1±3.1 and 13.3±3.5 years, respectively. At baseline 31.8% had no retinopathy, 67.4% had non-PDR and 0.8% had PDR.
At follow-up, the prevalence of diabetic retinopathy was 96.9%. Thirty-eight patients with no DR at baseline developed non-PDR in 16 years (76.2%). The 16-year incidence of PDR was 30.7%. Six patients (4.6%) went from having no DR at baseline to PDR at follow-up. In a crude analysis baseline AER (OR 4.3 versus patients without albuminuria, 95% CI 1.3-14.2) and HbA1c (OR 2.3 per 1%-point increase, 95% CI 1.5-3.8) were risk factors for incident PDR. In a multivariate analysis, however, baseline HbA1c was the only statistically significant risk factor for incident PDR. We were not able to find risk factors for incident DR due to the almost universal outcome.
Conclusions. Retinopathy among young Danish type 1 diabetic patients is almost universal. Despite the young age of the patients and the increased awareness of the importance of hyperglycaemia, in 16 years almost all patients developed retinopathy and one in three have progressed to PDR. Even for young type 1 diabetic patients, high levels of HbA1c drastically increases the risk of PDR.

NON-SWEDISH ORIGIN MAY BE A RISK FACTOR FOR DIABETIC RETINOPATHY AT DIAGNOSIS IN SCANIA, SWEDEN, IN 2008-2012

C. Gustavsson[1,2], M. Dorkhan[2], L. Groop[2]
[1]Department of Clinical Sciences in Malmö, Ophthalmology, Malmö, Lund University, Sweden; [2]Department of Clinical Sciences in Malmö, Diabetes and Endocrinology, Malmö, Lund University, Sweden

Introduction. Prospective population study.
Purpose. The regional program All New Diabetics in Scania (ANDIS) collects data from newly diagnosed diabetics from all hospitals and primary care centers in the county of Scania, Sweden, in order to establish up to date epidemiology and to define new risk factors for diabetes and its complications. ANDIS eye complications (ANDISec) evaluates diabetic retinopathy (DR) in 2000 subjects from the cohort. The DR prevalence at diagnosis in different ethnical subgroups is presented.
Methods. 2028 subjects from the ANDIS cohort went through routine fundus photography screening for DR. The International Classification of Diabetic Retinopathy was used for grading. Information on patient characteristics, diabetes type and ethnic origin was collected from the ANDIS database. Ethnic origin was defined as non-Swedish if at least one parent was born outside Sweden. Subgrouping of countries was performed according to the UN Country Classification System.
Results. Median age was 61 (17-91) years. 1180 were men (58.2%) and 848 women (41.8%). 84.5% had type 2 diabetes. 66.8% of subjects originated from Sweden. The largest single immigrant group was Iraq (3.6%). Southern Europe was the largest immigrant subgroup (7.6%) followed by Western Asia (6.5%), Northern Europe (5.5%) and Eastern Europe (4.5%). At diagnosis, 86.6% of subjects had no, 8.7% mild, 4,2% moderate, 0.4% severe and 0.1% proliferative DR. Immigrants were likelier to have DR at diagnosis (OR 1.3, p=0.019). The Iraqi cohort was at highest risk (OR 1.5, p= 0.016). 406 subjects (20%) attended a second visit. The DR prevalence at the second visit was also higher in the Iraqi than in the Swedish cohort (OR=1.7, p=0.028).
Conclusions. The prevalence of DR at diagnosis was low in the total population, perhaps reflecting early diabetes diagnosis as well as DR screening. However, the prevalence of DR both at diagnosis and at first follow up was higher among immigrants, than in subjects of Swedish origin. At this point, ANDISec does not give an explanation for these discrepancies, which might be genetic or related to socioeconomic factors.

STAGING OF DIABETIC RETINOPATHY UPON INITIAL PRESENTATION IN SAUDI ARABIA

R. M. Gandour[1], S. Waheeb[1,2,3], I. A. Alhabli[1], A. K. Baothman[1]
[1]Department of Ophthalmology, King Abdulaziz University, College of Medicine, Jeddah, Saudi Arabia; [2]Department of Ophthalmology, King Faisal Specialities Hospital, jeddah, Saudi Arabia; [3]Department of Ophthalmology, International Medical Center, Jeddah, Saudi Arabia

Introduction. The study was conducted as a cross-sectional survey of diabetic patients upon initial presentation to the retina clinic.
Purpose. Assessing the severity of this disease in our society will aid in imposing a national screening program to promote awareness, as well as limit debilitation of the quality of life amongst diabetic individuals.
Methods. The study was conducted as a cross-sectional survey of diabetic patients upon initial presentation to the retina clinic. Implementation of the study was based in four major hospitals in Jeddah: King Abdulaziz University Hospital, Jeddah Eye Hospital, King Faisal Specialist Hospital, and the International Medical Center. The designated sampling frame previously established includes both Saudi and non-Saudi residents, type 1 and 2 diabetic patients, and both genders of all ages.
Results. A sample of 443 patients was selected whilst targeting patients of all ages, gender, or type of diabetes. The number of patients presenting with diabetic retinopathy were 261, or 58.9% of the sample size. There was a significant direct relationship in relation to the duration of diabetes (p-value 0.001), as well as the severity of retinopathy. The prevalence was slightly higher among males (61.7%) in comparison to females (56.1%). Other medical conditions that showed significant relation to DR include nephropathy (p-value 0.001), hypertension (p-value 0.003), and hyperlipidemia (p-value 0.135). The results also showed a moderate increase of DR with increasing age (p-value 0.389).

Conclusions. The alarming numbers of this disease should be a cue for the initiation of a regular screening program in order to limit progression of the disease. Complications of diabetic retinopathy could be vision threatening and can cost patients their eyesight. This study aspires to prove the need of introducing an effective screening campaign in Saudi Arabia.

THE EFFECTS OF COMBINED THERAPY WITH METFORMINE AND SULFONYLUREA ON CLINICAL CHARACTERISTICS OF METABOLIC SYNDROME IN TYPE 2 DIABETES MELLITUS.

M. Carcani, S. Carcani, M. Keci
Service of Internal Medicine, Service of Cardiology Regional Hospital of Durres, Albania

Introduction. Insulin resistence is known to be the main underlying factor in development of metabolic syndrome. The aim of this study was to evaluate the effects of combined therapy with metformine and sulfonylurea in metabolic syndrome and diabetic retinopathy in patients with type 2 diabetes.
Methods. 50 patients diagnosed with type 2 diabetes and metabolic syndrome were treated with combined therapy of metformine and sulfonylurea for 6 months. Metformine dose was maximum (1500 mg/d) and sulfonylurea dose was 15 mg/d. Antihipertensive and hypolipemic therapy has not been changed in any of the patients. At the beginning of the study and after 6 months we evaluated basal (FBG) and postprandial glucose (PPG), HbA1c, body mass index (BMI), waist circumference, systolic and diastolic blood pressure, fasting plasma tryglicerides, total LDL, cholesterol and eye examination for diabetic eye disease.
Results. Glycemic control after six months of combination therapy was significantly improved (FBG 150 mg/dl vs 200 mg/dl $P<0,01$; PPG 230 mg/dl vs 270 mg/dl $P<0,01$; HbA1c 8,2% vs 10,5% $P<0,001$) BMI 28,5 vs 30 kg/m^2 $P<0,001$, and waist circumference 102 vs 110 cm $P<0,001$. Good results were achieved in fasting plasma triglycerides decreased to an average of 270 mg/dl from an average of 285 mg/dl $P<0,001$. At the beginning of the study, 30 out of 50 patients had diabetic retinopathy, of these 30, 20 patients had nonproliferative retinopathy and the remaining 10 patients had proliferative retinopathy; 6 months after combined treatment, no changes were seen regarding the diabetic eye disease.
Conclusion. Combined therapy with metformine and sulfonylurea presents better results than monotherapy regarding the possibility of decreasing the risk of cardiovascular disease and this is done through reduction of blood glucose values, plasma triglycerides, central obesity and BMI. On the other hand, combined therapy had no effect on diabetic eye disease.