EJO European Journal of Ophthalmology

European Journal of Ophthalmology
2022, Vol. 32(1S) 1–24

DOI: 10.1177/11206721221092575
journals.sagepub.com/home/ejo
SAGE

32nd Meeting of the European Association for Diabetic Eye Complications (EAsDEC) Belfast, Northern Ireland, United Kingdom, 26th – 28th May 2022

Free Paper Sessions

Association of Predominantly Peripheral Lesions on Ultrawide Field Imaging and the Risk of Diabetic Retinopathy Worsening Over Time: Results from the DRCR Retina Network Protocol AA

P.S. Silva[1] on behalf of the DRCR Retina Network

[1]*Joslin Diabetes Centre, Department of Ophthalmology, Harvard Medical School, Boston, MA, USA*

DESIGN OF STUDY. Prospective multicenter longitudinal observational study
PURPOSE. To determine whether predominantly peripheral lesions (PPL) identified on UWF imaging and angiographic risk factors are associated with increased disease worsening beyond the risk associated with baseline ETDRS DR severity score (DRSS).
METHODS. A total 544 study eyes with nonproliferative DR (NPDR) from 367 adult participants with diabetes. 200° UWF-colour images were collected at each annual visit through 4 years. UWF-fluorescein angiography (FA) images imaging was performed at baseline, 1 and 4 years. A centralized reading center graded DR severity and PPL on UWF-colour (colour-PPL) and UWF-FA (FA-PPL). PPL were defined as DR lesions with a greater extent outside versus inside the standard ETDRS fields. Initiation of treatment for DR and/or DME was at investigator discretion. The primary outcome was disease worsening over 4 years, defined as 2 or more steps DRSS worsening within ETDRS fields on UWF-colour images or receipt of DR treatment.
RESULTS. The 4-year disease worsening rates were 45% for eyes with mild NPDR at baseline, 40% for moderate NPDR, 26% for moderately-severe NPDR, and 43% for severe NPDR. At baseline, 41% of eyes had colour-PPL and 46% had FA-PPL. Disease worsening was not associated with baseline colour-PPL (present vs absent: 38% vs 43%; HR, 0.78; 95% CI, 0.57–1.08; $p = 0.13$) but was associated with baseline FA-PPL (present vs absent: 50% vs 31%; HR, 1.72; 95% CI, 1.25–2.36; $p < 0.001$).
CONCLUSIONS. Although no relationship was identified with colour-PPL, presence of FA-PPL was associated with greater risk of disease worsening over 4 years, independent of baseline DR severity level. These results suggest that evaluation of the retinal far periphery is important in understanding which eyes with NPDR are at higher risk for future disease worsening. Peripheral findings on UWF-FA should be considered for incorporation in future DR staging systems.

Characterization of Two-Year Progression of Neurodegeneration in Different Risk Phenotypes of Diabetic Retinopathy

A.R. Santos[1,2,4], C. Lobo[1,2,3], L. Ribeiro[1,2], I.P. Marques[1,2], S. Ferreira[1], T. Santos[1] and J. Cunha-Vaz[1,2]

[1]*AIBILI - Association for Innovation and Biomedical Research on Light and Image, Coimbra, Portugal*
[2]*Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, University of Coimbra, Coimbra, Portugal*
[3]*Department of Ophthalmology, Centro Hospitalar e Universitário de Coimbra (CHUC), Coimbra, Portugal*
[4]*Department of Orthoptics, School of Health, Polytechnic of Porto, Porto, Portugal*

DESIGN. Prospective observational 2-year study.
PURPOSE. To characterize the two-year progression of neurodegeneration in different diabetic retinopathy (DR) risk phenotypes in type 2 diabetes.
METHODS. A prospective longitudinal cohort study (CORDIS, NCT03696810) was conducted with 3 visits (baseline, 6-months and one-year). Demographic and systemic data included age, sex, diabetes duration, lipid profile and haemoglobin A1c (HbA1c). Ophthalmological examinations included visual acuity (BCVA), colour fundus photography (CFP) and optical coherence tomography (OCT and OCTA). Phenotype classification was performed, at 6-month visit, based on microaneurysm turnover (MAT on CFP) and central retinal thickness (CRT, on OCT). Only risk phenotypes B (MAT < 6 and increased CRT) and C (MAT ≥ 6 with or without increased CRT) were

included. ETDRS grading was performed at the baseline and last visit based on 7-fields CFP.

RESULTS. Of the 133 T2D individuals included in the study, 81 (60%) eyes were classified as phenotype B and 52 (40%) eyes as phenotype C. Of these, 127 completed the two-year follow-up, with 24 (19%) developing centre involving macular edema (CIME) and 2 (1.6%) clinically significant macular edema (CSME).

Neurodegeneration represented by thinning of the GCL +IPL was present in both phenotypes showing no statistically significant differences between these phenotypes. Furthermore, GCL+IPL thickness decreased with time (average of -0.605 µm/year; $p = 0.010$). This decrease remained statistically significant ($ß = 0.624$, $p = 0.006$) when controlling for age, sex, diabetes duration and HbA1c. Changes in time for GCL+IPL thickness are also negatively associated with longitudinal changes in FAZ area ($\beta = -1.469$), FAZ perimeter and deep capillary plexus vessel density in phenotype C and in contrast with Phenotype B. No correlation was found between the presence of increased neurodegeneration and the development of CIME.

CONCLUSIONS. In the two-year period of follow-up both phenotypes B and C showed progression in retinal neurodegenerative changes in phenotype C. The neurodegeneration is associated with microvascular related variables indicative of capillary closure. There is no association between the progression in neurodegeneration and development of CIME.

Transcriptomic Analysis Reveals that Retinal Neuromodulation is the Main Underlying Mechanisms of the Neuroprotective Effect of Sitagliptin in Diabetic Retina

H. Ramos[1], P. Bogdanov[1,2], J. Huerta[1], D. Sabater[1], A. Deàs-Just[1], C. Hernández[1,2,3] and R. Simó[1,2,3]

[1]*Diabetes and Metabolism Research Unit, Vall d'Hebron Research Institute, Barcelona, Spain*
[2]*Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Instituto de Salud Carlos III (ICSIII), Madrid, Spain*
[3]*Department of Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain*

DESIGN. Neurovascular unit (NVU) impairment is an early event in the pathogenesis of diabetic retinopathy (DR), which participates in the neurodegeneration and the early microvascular impairment of the diabetic retina. Consequently, NVU becomes an emergent therapeutic target of DR. The diminishment of synaptic protein expression, the impairment of neurotransmission and alterations in neuronal morphology have been described as underlying mechanisms of NVU impairment. Topical administration (eye drops) of sitagliptin, dipeptidyl peptidase-4 inhibitor (DPP-4i), prevented retinal neurodegeneration induced by diabetes in db/db mice.

PURPOSE. To further explore the mechanisms involved in the beneficial effects of DPP-4i on diabetes-induced retinal neurodegeneration, we have compared the retinal expression patterns of vehicle-treated db/db mice (an experimental model of DR) with db/db mice treated with sitagliptin.

METHODS. Ten db/db mice, aged 10 weeks, were topically treated with sitagliptin eye drops (5 µL/eye; concentration: 10 mg/mL) for 2 weeks twice per day, while other ten db/db were received a topical administration of vehicle (5 µL/eye). Ten db/+ mice (non-diabetic mice) were assigned as control group. At 12 weeks, after euthanasia, one eye was used for a transcriptomic analysis and the other for its validation through RT-PCR and for protein assays through Western Blotting (WB) and Immunohistochemistry (IHC). Full-field electroretinogram recordings were used to address retinal functionality.

RESULTS. Diabetic mice topically treated with sitagliptin presented different expression patterns in the retina in comparison to those treated with vehicle. In the analysis of biological significance, neurotransmission was the most enriched biological process. Additionally, we observed that both mRNA and WB/IHC of presynaptic proteins involved in vesicle biogenesis, mobilization, docking, fusion and recycling, were down-regulated in db/db mice retinas in comparison with non-diabetic controls. Topical administration of sitagliptin inhibits this down-regulation caused by diabetes and improves the functionality of diabetic retinas. This effect was unrelated to blood glucose improvement.

CONCLUSIONS. Sitagliptin exerts neuroprotective effects in db/db mice retinas by inhibiting the diabetes-induced down-regulation of key presynaptic proteins. This finding open up a new strategy for treating not only DR but also other retinal diseases in which synaptic abnormalities/neurodegeneration play a crucial role.

Inflammation is Underlying in Retinal Degenerations: Similarities and Differences Between Diabetic Retinopathy and Retinal Degeneration in Huntington's Disease

F. Cano-Cano[1], L.M.V. Valor[2], I.H.C. Hervás-Corpión[3], A.G.O. Gallardo-Orihuela[1], L.G.J. Gómez-Jaramillo[1], M.C.G.M. González-Montelongo[1] and A.I.A. Arroba[1]

[1]*Diabetes Laboratory, Research Unit, Biomedicine Research and Innovation Institute of Cadiz (INiBICA), Puerta del Mar University Hospital, Cádiz, Spain*
[2]*Research Support Laboratory, Alicante University Hospital, Institute of Sanitary and Biomedical Research of Alicante (ISABIAL), Alicante, Spain*
[3]*The Advanced Therapies for Pediatric Solid Tumors Research Group, Clínica Universidad de Navarra - Centro de Investigación Médica Aplicada (CIMA), Navarra, Spain*

DESIGN. As in Diabetes Mellitus, there are increasing evidence indicates the presence of abnormal glucose tolerance and hyperglycaemia in Huntington's Disease (HD). Besides, there is a structural and functional affectation in the retinas of this patient. Nevertheless, the relation of the hyperglycemia and retinal affectation have not been fully studied. The present work explores the retinal alterations linked to the disease in an animal model of HD R6/1 strain, which is known for present blood glucose alterations, in order to define the similar characteristics between diabetic retinopathy (DR) and retinal degeneration during HD.
PURPOSE. The aim of this study was to investigate the retinal alterations present in R6/1 HD mouse model and determine the similarities and differences with DR events.
METHODS. Retinal function and structure was measure in R6/1 and wild type (WT) mouse at 7, 13 and 24 weeks of age. The retinal function was measure by electroretinogram (ERG) at 24 weeks. Retinal structure was measure through ocular coherence tomography (OCT) and immunofluorescence. Neuro-inflammatory pathways were analysed by qPCR and western blotting and RNA sequencing.
RESULTS. In an early state at 7 weeks, R6/1 present ramified and amoeboid microglia in IPL and GCL layers. There is an increase presence of gliosis in ganglion cell layer (GCL) and outer plexiform layer (OPL) at 7 weeks and later gliosis in INL and IPL at 13 weeks. Also, the expression of photoreceptor cells genes are down-regulated and non-neuronal cells genes, like microglia, are up-regulated at this age. Visual function of HD mouse model shows a disruption in a- and b- waves at 25 weeks and there is no reduction of retinal thinning and loss of retinal structure.
CONCLUSIONS. These data suggest that HD involves a progressive decline of retinal and structural function. R6/1 mouse model present a delimited inflammatory response characterized by gliosis and microglia activation as occurs during DR in several animal models. Furthermore, there is an alteration in ERG pattern but, differently to DR models, there is no reduction of retinal thinning.

Retinal Neurovascular Dysfunction in a Mouse Model of Alzheimer's Disease Combined with Type 2 Diabetes

K. Little[1,2], M. Llorián-Salvador[1], Á. del Marco[2], M. Garcia-Alloza[2], R. Simó[3] and A. Stitt[1]

[1]*Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK*
[2]*Division of Physiology, School of Medicine, University of Cadiz, Cadiz, Spain*
[3]*Vall d'Hebron University Hospital, Barcelona, Spain*

DESIGN. Experimental.
PURPOSE. Type-2 diabetes (T2D) is associated with an increased risk of cognitive impairment and Alzheimer's disease (AD). Common pathways involved in T2D and AD include alterations of the neurovascular unit (NVU). Retinal NVU degeneration is an important early event in the development of diabetic retinopathy (DR). The retina is proposed to be an easily accessible window to the brain, therefore retinal pathology may be useful to understand cognitive changes in T2D. This study assessed retinal pathology in mice models of AD and T2D.
METHODS. The NVU was assessed in retinal tissue from WT, APP/PS1, db/db and APP/PS1 × db/db mice at 14 and 26 weeks old. Immunohistochemistry staining was carried out to assess gliosis (GFAP) and acellular capillaries (Isolectin-B4/Collagen 4). In addition, changes to neuronal populations were assessed by staining for Calbindin (Horizontal cells), Brn3a (Retinal Ganglion cells) and Cone-arrestin (Cone-photoreceptors).
RESULTS. We observed evidence of NVU dysfunction in the retina of APP/PS1 × db/db mice including significant Müller cell gliosis at 14 weeks ($p < 0.05$). APP/PS1 × db/db mice had significantly more acellular capillaries than WT mice at 14 weeks ($p < 0.01$). A significant decrease in retinal ganglion cells was observed at 26 weeks in db/db ($p < 0.05$) and APP/PS1 × db/db mice ($p < 0.05$) compared to WT. Horizontal cells were significantly reduced in APP/PS1 ($p < 0.05$) and APP/PS1 × db/db ($p > 0.05$) retina at 26 weeks. A significant decrease in the number of Cone arrestin+ cells in the APP/PS1 × db/db retina was observed at both 14 ($p < 0.001$) and 26 weeks ($p < 0.001$) vs WT. The number of DAPI+ cells in the outer nuclear layer was significantly reduced in APP/PS1 × db/db mice compared to APP/PS1 ($p < 0.001$) and db/db ($p < 0.05$) alone.
CONCLUSIONS. We observed evidence of NVU dysfunction in the retina of APP/PS1 × db/db mice, which appeared to be more severe than APP/PS1 or db/db alone. This occurs alongside severe cognitive impairment in this model. APP/PS1 × db/db had increased gliosis and retinal neurodegeneration features. Further studies are required to characterise the changes of the NVU in the retina and brain during diabetes related neurodegeneration.

Investigation of the Heterocellular Features of the Retinal Neurovascular Unit using 3D Electron Microscopy

M. J. Albargothy[1,2], N.N. Azizah[1], S.L. Sarah[2], E.P. Troendle[2], D.H.W. Steel[1], T.M. Curtis[2] and M.J. Taggart[1]

[1]*Biosciences Institute, Newcastle University, International Centre for Life, Newcastle upon Tyne, UK*
[2]*Wellcome Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK*

DESIGN. Serial-block face scanning electron microscopy (SBF-SEM) and computational image reconstruction was used to provide the first 3-dimensional ultrastructural analysis of the mouse retinal neurovascular unit (NVU).

PURPOSE. Disruption of the integrity of the retinal NVU has been implicated in the pathogenesis of diabetic retinopathy (DR). Despite this, the 3-dimensional ultrastructure of the retinal NVU remains to be fully characterized and quantitative methods for describing its key features have yet to be developed. In the present study, we have undertaken the first nanoscale examination of the anatomy of the mouse retinal NVU in three spatial dimensions using SBF-SEM.
METHODS. Resin-embedded mouse retinal tissue samples were imaged using a Zeiss Sigma SEM chamber combined with Gatan 3View software. For capillaries located in the superficial plexus, the vascular basement membrane and cells of the retinal NVU were segmented using Microscopy Image Browser (MIB). Segmented features were visualized and rendered in 3D using Amira or ARIVIS software. Cellular morphologies and cell proximities were quantified using custom-written scripts in MATLAB r2021b.
RESULTS. Prominent feature of the capillary arrangements in 3D was the extensive sheath-like coverage by singular pericytes. They appeared in close register to the basement membrane with which they interwove in a complex mesh-like appearance. Breaks in the basement membrane appeared to facilitate pericyte interactions with other NVU cell types. There were frequent, close (<10 nm) pericyte to endothelial interactions with direct contact points and peg-and-socket-like morphology. Macroglia typically intervened between neurons and capillary structures; however, regions were identified where neurons came into closer contact with the basement membrane. A software generated analysis to assess the morphology of the different cellular components of the NVU, including quantifications of convexity, sphericity, and cell-to-cell closeness, has enabled preliminary semi-quantitative characterization of cell arrangements with neighbouring structures.
CONCLUSIONS. This work provides new information on the heterocellular and cellular-basement membrane features of the murine retinal NVU. It also serves as a platform to inform future studies examining changes in NVU characteristics during the onset and progression of DR.

Differential Roles of eNOS in VEGF-Induced Permeability in Barrier Versus Non-Barrier Microvascular Endothelial Cells

E.K. Bosma[1], Y.I. Habani[1], I.M.C. Vogels[1], C.J.F. Van Noorden[1,2], R.O. Schlingemann[1,3] and I. Klaassen[1]

[1]*Department of Ophthalmology, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands*
[2]*Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Ljubljana, Slovenia*
[3]*Department of Ophthalmology, University of Lausanne, Jules Gonin Eye Hospital, Fondation Asile Des Aveugles, Lausanne, Switzerland*

DESIGN. Fundamental research study with cultured primary endothelial cells (ECs)
PURPOSE. Vascular endothelial growth factor (VEGF) is a key signaling molecule involved in the barrier function of the endothelium. When its expression is elevated in the retina, VEGF induces permeability, which may ultimately lead to vision-threating conditions such as diabetic macular edema. Endothelial nitric oxide synthase (eNOS) has previously been identified to regulate VEGF-induced endothelial permeability. However, the molecular pathways remain incompletely understood. In this study, we set out to investigate the role of eNOS in VEGF-induced permeability in non-barrier ECs and barrier ECs, focusing on the transendothelial transport pathway.
METHODS. Human dermal microvascular endothelial cells (HDMVECs; non-barrier like ECs), human retinal microvascular ECs (HRECs; barrier-like ECs) and bovine retinal ECs (BRECs; barrier-like ECs) were used for experiments. To investigate the role of eNOS, cells were treated with siRNA against eNOS to reduce eNOS expression, with the NOS inhibitor L-NAME to inhibit eNOS activity, or with the NO donor SNAP. The barrier function was studied with a Transwell permeability assay using different tracers (70 kDa dextran-FITC, BSA-FITC and 766 da Cy3 tracer). In addition, dextran and BSA tracer uptake was studied in fixed cells with microscopy. Protein and gene expression was analyzed by western blotting, immunocytochemistry and qPCR.
RESULTS. Silencing of eNOS expression reduced VEGF-induced permeability for dextran and 766 Da-tracer in HDMVECs, but not in HRECs. On the other hand, silencing of eNOS expression in HRECs increased permeability to dextran, BSA and 766 Da-tracer in the absence of VEGF stimulation, suggesting a protective function of eNOS in resting HRECs. eNOS negatively regulated dextran uptake in HDMVECs and HRECs, but not in the presence of VEGF. In contrast, BSA uptake was not dependent on eNOS in HDMVECs, whereas in HRECs it is negatively regulated by eNOS. Finally, we demonstrated that eNOS regulates expression of the caveolae-associated protein PLVAP in HDMVECs, but not in HRECs.
CONCLUSIONS. Our findings demonstrate that eNOS has differential roles in the regulation of endothelial transcytosis and permeability in barrier and non-barrier endothelium.

Single Cell Analysis of the Retina to Elucidate Early Changes in Diabetes

S. Bridgett[1], O. Cappa[1], E. Spencer[1], T. Friedel[1], T. Curtis[1] and D.A. Simpson[1]

[1]*Wellcome–Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK*

DESIGN. Diabetic retinopathy (DR) is a leading cause of blindness globally and more effective treatments aimed at

the earlier stages of the disease are required. Although often considered primarily a vascular disease, there is evidence of early glial and neuronal dysfunction. The aim of the study is to determine the early diabetic changes in each of the retinal cell types. Using a murine model of diabetic retinopathy control and diabetic retinas are compared using single-cell RNA sequencing (scRNA-Seq).
PURPOSE. To measure the effect of diabetes upon the transcriptomes of the individual cell types of the retina.
METHODS. Control and treated retinas from a murine model of streptozotocin-induced diabetes (3 mice each group) were collected after 3, 19 and 26 weeks of diabetes and dissociated into single cells. scRNA-Seq was performed using the 10X Genomics and Illumina platforms. Sequencing data was processed using the Seurat package in R to define cell types and perform differential gene expression.
RESULTS. Over 30,000 thousand cells, representing all the main cell types, were profiled from control and diabetic retinas. No significant changes in cell proportions were observed, but diabetes caused significant differential gene expression in most cell types. Prominent changes in protein synthesis and oxidative phosphorylation occurred in many cells. Expression of histones, including H3f3b increased in several cell types and time points. Muller cells showed an increase in metallothionenin genes, potentially offering protection against oxidative stress.
CONCLUSIONS. This study has confirmed early molecular changes in neural and glial cells during diabetes and as expected, suggested changes within vascular cells. Enrichment of the relatively rare endothelial cells and pericytes will be required to provide more conclusive data regarding vascular effects. We have identified many novel potential targets for early interventions to prevent or slow diabetic retinopathy.

Circadian Rhythms in Diabetic Retinopathy

H. Winter[1], T. Friedel[1], J. Augustine[1], A.W. Stitt[1] and E. Beli[1]

[1]*Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK*

DESIGN. Activity rhythms, as an output of the master circadian clock, and rhythms of electroretinograms (ERG), as an output of the retinal circadian clock, were measured in mouse models of type 1 diabetes.
PURPOSE. Diabetes disrupts circadian rhythms and circadian disruption, in turn, plays an emerging role in disease prognosis. The retina is an integral part of the pathway through which light entrains the master circadian clock via the retino-hypothalamic track, and so the goal of this study was to reveal whether changes in the retina due to diabetes might impact activity.
METHODS. Changes in master clock outputs were investigated in 6-month-old Ins2AKITA mice ($n = 8$) and C57BL/6J controls ($n = 4$) housed in normal 12L:12D lighting (200lux). Wheel running activity was analysed for markers of circadian disruption. Retina function was measured at 8am, 2pm, 8pm and 2am ($\geq n = 5$ mice per time point) after 24 h dark adaptation using ERG. Flicker ERG was also compared between 6-month-old diabetic ($n = 6$) and wild type mice ($n = 9$).
RESULTS. Circadian disruption is apparent in diabetic mice by 6 months compared to controls, including increased light phase activity ($17.3 \pm 8.0\%$ vs $9.8 \pm 5.6\%$), decreased inter-daily stability (0.5 ± 0.1 vs 0.8 ± 0.1), and increased intra-daily variability (1.0 ± 0.3 vs 0.6 ± 0.1). Significant differences in ERG markers such as the amplitude of oscillatory potentials are detected in 6-month-old diabetic mice. Further, using JTK_Cycle analysis, we detect rhythmicity in the b-wave amplitude of dark-adapted ERG in wild type mice only ($p = 0.02$) that appears to be lost in diabetes ($p = 0.50$). No differences were observed in flicker ERG amplitudes.
CONCLUSIONS. Circadian rhythms in both the master circadian clock and the retina clock are altered in diabetes. Circadian disruption in locomotor activity worsens with disease duration, as exemplified by abnormal light phase activity in 6-month-old diabetic mice. Diabetic mice lack the rhythmicity in ERG b-wave amplitude seen in wild type mice, indicating a disruption in the retina clock affecting the inner nuclear layer. More research is required to elucidate the defects of the intrinsic and extrinsic inputs to ipRGCs that entrain the master clock.

Extracellular Vesicles Derived from M1-Activated Microglia Induce Pro-Retinopathic Functional Changes in Microvascular Cells

E. Beltramo[1], A. Mazzeo[1] and M. Porta[1]

[1]*Department of Medical Sciences, University of Turin, Turin, Italy*

DESIGN. Experimental study aimed at understanding the interactions between microglia and vascular cells in the diabetic retina.
PURPOSE. Microglial cells are the main responsible for the modulation of the immune response to inflammatory stimuli inside the diabetic retina. Most studies addressing microglial potential to modulate diabetic retinopathy use rodent cells. An immortalized human microglial line, tested to verify its susceptibility to inflammation, is now available and represents a potential tool to investigate the pathophysiology of the inflammatory component of diabetic retinopathy in species-specific models. Extracellular vesicles (EVs) carry several molecules (miRNAs, mRNAs, proteins) and are known to exert a paracrine function in the transmission of signals between neighbouring tissues. Our aim was to characterize EVs released by

microglial cells in inflammatory conditions and investigate their effects on the vascular components of the retina.
METHODS. Human microglial cells were exposed for 24 h to an inflammatory cocktail (20 ng/mL hIL − 1β + 10 ng/ml hTNFα + 50 ng/mL hINFγ), able to induce M1 pro-inflammatory polarization. 50 μmL/L thiamine were also added, to test the anti-inflammatory potential of this vitamin. EVs were isolated from the supernatants and characterized by Nanosight and Western blotting. EV content in miRNAs and mRNAs was investigated. Human retinal endothelial cells (HRECs) and pericytes (HRPs) were exposed for 24 h to microglial-derived EVs and their response in terms of proliferation, apoptosis, migration, and ROS production assessed.
RESULTS. All EVs isolated from the supernatants of microglial cells showed characteristic EV markers (Alix, CD63, CD81). Pro-inflammatory miR-21 and miR-155, as well as CCL-2 and MMP-2 expression, were increased in M1 microglia-derived EVs, and normalized by the addition of thiamine to microglia cultures. M1-derived EVs increased apoptosis, migration, and ROS production in both HRPs and HRECs, and HRP proliferation. Addition of thiamine normalized all these parameters.
CONCLUSIONS. EVs derived from M1-activated microglia can stimulate functional changes in microvascular cells and induce features of retinopathy. Thiamine exerts an anti-inflammatory protective effect, when added to M1 microglial cultures.

Proteomics Analysis of Ocular Tissues from Donors with Diabetes and Diabetic Retinopathy

P.E. Fort[1], F. Da Veiga Leprevost[1], A. Nesvizhskii[1] and H. Rachana[1]

[1]*University of Michigan, Ann Arbor, MI, USA*

DESIGN. After identification and classification of 40 donors in 4 groups (10 per group): No diabetes mellitus (ND), With diabetes but without retinopathy (D), those with mild retinopathy (mDR) and those with severe non-proliferative retinopathy (adDR). Following qualitative controls, Tandem Mass Tag (TMT) proteomic analysis was performed on a fragment of the peripheral and peri-macular (central) region of the retina as well as vitreous humor from these 40 donors through the University of Michigan (UM) Pathology Department Proteomic Core. Differential expression and pathway analysis were then performed with the UM Computational Medicine and Bioinformatics group, prior to further validations.
PURPOSE. Despite continuous efforts and some recent progress regarding treatment of late stages, DR continues to be a major public health problem, in part due to incomplete understanding of its pathophysiology. There is a persistent knowledge gap regarding mechanisms that damage the neurovascular unit in DR preventing identification of actionable pathways in humans. Our overall goal is to better understand the pathophysiological mechanisms involved in the onset and progression of DR, in order to diagnose, prevent and treat early stages of the disease. The general hypothesis is that alterations of the retinal and vitreous humor proteome partially correlate and are reflective of the pathophysiology of diabetic retinopathy and its progression.
METHODS. The major goal was to characterize the retinal and vitreal proteome of 40 samples classified in 4 groups (10 per group) as described above: ND / D / mDR and adDR by TMT analysis.
RESULTS. More than 7600, 7800 and 3090 individual proteins were identified and quantified in the individual samples of the central retina, peripheral retina and vitreous humor respectively. Of those more than 1300, 790, and 240 individual proteins were differentially expressed in the central retina, peripheral retina and vitreous humor of the severe NPDR groups respectively, when compared to those without diabetes. Among the top biological processes and pathways identified as significantly different were pathways associated with inflammation such as the complement system, as well as pathways involved in neurovascular unit homeostasis.
CONCLUSIONS. The current study demonstrates the potential of human based proteomics studies for the understanding of the onset and progression of DR, including relative to the relationship between retinal and vitreous makeup.

Diabetic Macular Oedema and Diode Subthreshold Micropulse Laser (Diamonds)

N. Lois[1] on behalf of the DIAMONDS Study

[1]*Wellcome Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK*

DESIGN. Randomised trial.
PURPOSE. Present clinical-effectiveness, safety, and cost-effectiveness of subthreshold micropulse laser (SML), compared with standard threshold laser (SL), for the treatment of people with center-involving diabetic macular edema (CI-DME) with central retinal thickness (CRT) <400μ.
METHODS. Participants: Adults with <400μ CI-DME and best-corrected visual acuity (BCVA) >24 Early Treatment Diabetic Retinopathy Study (ETDRS) letters in one/both eyes.
Intervention: Randomisation 1:1 to 577 nm SML or SL; retreatments were allowed. Rescue with intravitreal anti-vascular endothelial growth factor therapies or steroids was permitted if >10 ETDRS-letter-loss and/or CRT increased >400μ.

Outcomes: Primary: Mean change in BCVA in the study eye at 24 months (non-inferiority margin 5 ETDRS-letters). Secondary: mean change from baseline to month-24 in binocular BCVA; CRT and mean deviation (MD) of Humphrey 10-2 visual field in the study eye; percentage meeting driving standards; EuroQoL (EQ-5D-5L), National Eye Institute Visual Function Questionnaire (NEI-VFQ25), and Vision and Quality of Life Index (VisQoL) scores; cost per quality-adjusted life years (QALY) gained; adverse effects; number of laser treatments; rescue treatments.

RESULTS. DIAMONDS recruited fully (n = 266); 87% SML and 86% SL had primary outcome data.

Mean BCVA change from baseline to month-24 was −2.43 (Standard Deviation [SD] 8.20) and −0.45 (SD 6.72) in SML and SL, respectively. SML was deemed not only non-inferior but also equivalent to SL as the 95% confidence interval (CI) (−3.9 to −0.04) lied wholly within both upper and lower margins of the permitted maximum difference (5 ETDRS-letters). There was no statistically significant difference in binocular BCVA (0.32 ETDRS letters; 95% CI −0.99, −1.64; $p = 0.63$), CRT (−0.64 microns; 95% CI −14.25, −12.98; $p = 0.93$), MD (0.39 dB; 95% CI −0.23, −1.02; $p = 0.21$), meeting driving standards (% point difference 1.6, 95% CI −25.3, 28.5; $p = 0.91$), side effects (Risk Ratio 0.28, 95% CI 0.06, 1.34; $p = 0.11$), rescue treatments (% point difference −2.8, 95% CI −13.1, 7.5; $p = 0.59$) or EQ-5D/VFQ-25/VisQoL scores. Number of lasers was higher in SML (0.48; 95% CI 0.18, 0.79; $p = 0.002$). Base-case analysis indicated no differences in costs/QALYs.

CONCLUSIONS. SML was equivalent to SL, requiring slightly higher number of laser treatments.

Fluctuations in Diabetic Retinopathy Severity: Associated Factors and Prognostic Significance

M.V. Cicinelli[1], G.G. Gregori[1], B.T. Tombolini[1], C.B. Barresi[1], R.L. Lattanzio[1] and F.B. Bandello[1]

[1]*San Raffaele Scientific Institute, Milan, Italy*

DESIGN. Longitudinal retrospective cohort study

PURPOSE. To longitudinally investigate the factors associated with diabetic retinopathy (DR) severity fluctuations and to explore their prognostic implications in terms of progression to proliferative DR (PDR).

METHODS. Patients with DR having >=2 ultra-widefield (UWF) fundus photography examinations and no history of PDR-related complications at baseline visit were included. UWF fundus images were graded using the Early Treatment Diabetic Retinopathy Study severity scale (DRSS). Multivariable linear mixed models were used to assess factors associated with DRSS standard deviation (SD) as a proxy of fluctuations. Risk factors for DR improvement or progression were computed with mixed-effect Cox hazard models. The mean DRSS area-under-the-curve (AUC) was included as a covariate in all statistical analyses.

RESULTS. 111 eyes with a median follow-up of 44 months and a median of 3 (IQR 2-5) UWF fundus photography exams per study eye were included. Type 1 diabetes (estimate[95% confidence interval(CI)] = 4.36[0.77–7.91] SD DRSS, $p = 0.02$) and macular non-perfusion (estimate[95% CI] = 5.30[1.70–8.72] SD DRSS, $p = 0.004$) were associated with wider fluctuations in DR severity. During the follow-up, 89 eyes (73%) underwent intravitreal injections for macular edema; higher number of anti-vascular endothelial growth factor injections administered (hazard ratio (HR)[95% CI] = 1.15[1.01–1.33] for injection, $p = 0.03$) was associated higher chance of ≥1-step improvement in DRSS. Higher HbA1c (HR[95% CI] = 1.83[1.26–2.69] for 1% increase, $p = 0.01$), higher mean DRSS-AUC (HR[95% CI] = 1.25[1.10–1.43] for DRSS/month increase, $p < 0.001$), and being in the 4th quartile of DR fluctuations (HR[95% CI] = 10.3[2.30–45.9] vs. 1st-3rd quartile, $p = 0.002$) were risk factors for PDR.

CONCLUSIONS. Patients with larger DR variability may need attentive follow-up to early identify DR worsening. DRSS-AUC may be a useful prognostic factor for future studies.

Evaluation of Intravitreal Aflibercept Treatment Effectiveness in UK Routine Clinical Practice for Patients with Diabetic Macular Edema: 24-month Outcomes of the DRAKO Study

J. Talks[1], S. Sivaprasad[2], S.P. Kelly[3], F. Ghanchi[4], A. Kotagiri[5], M. Saddiq[6] and P. Scanlon[7]

[1]*Newcastle Upon Tyne Hospitals NHS Foundation Trust, Newcastle Upon Tyne, UK*
[2]*National Institute for Health Research, Moorfields Biomedical Research Centre, London, UK*
[3]*Bolton Hospital NHS Foundation Trust, Bolton, UK*
[4]*Bradford Teaching Hospitals NHS Foundation Trust, Bradford, UK*
[5]*South Tyneside and Sunderland NHS Foundation Trust, Sunderland, UK*
[6]*O4 Research Limited, Belfast, Northern Ireland, UK*
[7]*Gloucestershire Hospitals NHS Foundation Trust, Gloucester, UK*

DESIGN. DRAKO (NCT02850263) is a prospective, observational study.

PURPOSE. To evaluate the effectiveness of intravitreal aflibercept (IVT-AFL) treatment in patients with diabetic macular oedema (DMO) within UK routine clinical practice over 2 years.

METHODS. Treatment-naïve patients (C1) and patients with prior anti-vascular endothelial growth factor treatment for DMO other than IVT-AFL (C2) meeting the study criteria were enrolled from 35 National Health Service centres

across the UK. Patients were treated according to local practice. Clinical outcomes, monitoring assessments and treatment practices were recorded. Primary endpoints were mean change from baseline in best-corrected visual acuity (BCVA) and central subfield thickness (CST) at Month 12 (M12). Secondary endpoints included mean change from baseline in BCVA and CST by Month 24 (M24) and diabetic retinopathy (DR) grade and haemoglobin A1c (HbA1c) across the study term. Data were analysed for patients with BCVA or CST outcomes available at baseline and M12 or M24 within a ±1-month window and summary statistics were generated for each endpoint. Analyses at M12 and M24 included: C1, 388 and 326; C2, 169 and 135 patients respectively.

RESULTS. Mean changes from baseline in BCVA and CST at M12 were: C1, +2.5 letters and −119.1 μm; C2, +0.2 letters and −79.1 μm, respectively. At M24, the mean changes from baseline were: C1, +0.7 letters and −123.3 μm; C2, −0.3 letters and −91.6 μm, respectively. DR grade and HbA1c outcomes from baseline were relatively stable across the 2-year period for both cohorts. The mean number of injections over 2 years was 8.9 for C1 patients and 9.3 for C2 patients. The safety profile of IVT-AFL was consistent with previous studies. Overall, 2 (0.3%) patients reported endophthalmitis, and there were no reports of intraocular inflammation or retinal vasculitis.

CONCLUSIONS. The DRAKO study indicates that IVT-AFL is an effective treatment for patients with DMO in routine clinical practice, despite patients receiving fewer injections than recommended in the summary of product characteristics. CST continued to decrease over the follow-up period and visual acuity was stable at M24 for both cohorts assessed.

Real-world Cohort of Patients with Diabetic Macular Oedema from 4 UK National Health Service Treatment Centres

T. Peto[1], F. Ghanchi[2], S.J. Talks[3], G. Menon[4], X. Chen[5] and G. Chin[5]

[1]*Centre for Public Health, Queen's University Belfast, Belfast, UK*
[2]*Bradford Teaching Hospitals NHS Foundation Trust and Bradford Ophthalmology Research Network, Bradford, UK*
[3]*Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK*
[4]*Frimley Health NHS Foundation Trust and NIHR Kent Surrey Sussex Clinical Research Network, Frimley, UK*
[5]*Genentech, Inc., South San Francisco, CA, USA*

DESIGN. Prospective clinical cohort.

PURPOSE. Intravitreal anti–vascular endothelial growth factor (VEGF) injections are effective in treating Diabetic Macular Oedema (DMO), but real-world outcomes are discordant when optimal monitoring and injection pathway are considered. We assessed cohort characteristics and visual acuity (VA) outcomes in patients with DMO receiving anti-VEGF injections in routine clinical practice from 4 national health service units in the UK between 2014-2019.

METHODS. Anonymised socio-demographic and clinical data (including visual acuity reported in Early Treatment Diabetic Retinopathy Study [ETDRS] letters) were extracted from Medisoft electronic medical records. One study eye per adult patient with DMO was included (for bilateral patients the first eye to meet the DMO case definition, or the right eye if both eyes met the definition simultaneously). Included eyes had ≥1 anti-VEGF injection and ≥180 days of follow-up.

RESULTS. This cohort includes 2074 patients with mean follow-up of 3.5 years, mean age 62.6 years at index treatment. The sample was 39.9% female, 81.6% White, and 80.2% had type 2 diabetes. Overall, 28.0% of patients had bilateral disease at baseline, 20.9% developed bilateral disease during the study period, and 51.2% remained unilateral. Mean (SD) baseline VA was 61.0 (15.6) letters. In the first 6 months, mean (SD) VA change from baseline was 4.4 (10.3) letters, and patients received a mean (SD) of 3.7 (1.4) anti-VEGF injections.

CONCLUSIONS. DMO patients included in this cohort were older adults, mostly White and with type 2 diabetes. Study eye VA improved on average approximately 4 letters 6 months after initial treatment with anti-VEGF.

Outcomes and Complications of Pars Plana Vitrectomy for Tractional Retinal Detachments in People with Diabetes Mellitus: A Systematic Review and Meta-Analysis

P. McCullough[1], A. Mohite[2], G. Virgili[2,3] and N. Lois[1,3]

[1]*Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, Northern Ireland, UK*
[2]*Department of Ophthalmology, The Belfast Health and Social Care Trust, Belfast, Northern Ireland, UK*
[3]*Centre for Public Health, Queen's University Belfast, Belfast, Northern Ireland, UK*

DESIGN. Systematic review and meta-analysis.

PURPOSE. Tractional retinal detachment (TRD) occurs in ~5% of people with proliferative diabetic retinopathy and, even with adequate treatment with PRP, it poses a threat to vision. Pars plana vitrectomy (PPV) is the treatment of choice for TRD. The purpose of this study was to determine anatomical and functional outcomes of PPV for the treatment of TRD in people with diabetes mellitus (dTRD).

METHODS. Medline and EMBASE were searched systematically from 1st Jan 2000 to 31st May 2021. Reference list of eligible studies were screened. Eligible

studies were those who reported outcomes of PPV for dTRD, published in English, including n > 25 eyes, and with a minimum follow-up of 3 months. We followed PRISMA guidelines for data extraction/synthesis and the National Institute for Health quality assessment tool to assess risk of bias. Study eligibility was determined independently by two reviewers; data extraction was conducted by one and entries checked for accuracy by another. Data were pooled using a random-effects model. Main outcomes included final visual acuity (VA) and rate of failure of retinal re-attachment after one surgery. Secondary outcomes included effect of baseline patient characteristics and surgical variables on postoperative outcomes.

RESULTS. Of 383 studies identified, 36 (3720 eyes) were eligible and included. The overall failure rate of retinal reattachment after one surgery was 5.9% (95% confidence interval [CI] 1.4–8.3%) and the final VA 0.94 LogMAR (~6/53 Snellen) (95% CI 0.81–1.07 LogMAR) (~6/39–6/71 Snellen). On multivariable analysis, no patient characteristics or surgical variables had a statistically significant effect on outcomes.

CONCLUSIONS. PPV is an effective strategy to achieve retinal re-attachment in people with dTRD. However, the final post-operative VA is low. Thus, patients should be counselled on the guarded prognosis of dTRD. Improvements in the surgical approach to dTRD and the development of early stage therapies for the prevention of dTRD are very much needed.

Utilization of Dilated Smartphone Fundus Imaging for Screening of Diabetic Retinopathy in an Urban Community Setting in Pasig City, Philippines

K.L.R. Locaylocay[1], P.S. Silva[1,2], S.O. Valero[1] and V.L. Caparas[1]

[1]*Eye and Vision Institute, The Medical City, Manila, Philippines*
[2]*Joslin Diabetes Center, Harvard Medical School, Boston, MA, USA*

DESIGN. This is a community-based, prospective, comparative study.

PURPOSE. This study aims to determine the diagnostic efficacy of dilated smartphone fundus imaging in the detection and grading of diabetic retinopathy (DR) among patients with diabetes mellitus (DM) in an urban community setting.

METHODS. This study was approved by the Institutional Review Board of The Medical City. A sample size of 34 eyes were calculated based on the kappa statistic. Twenty-three adult DM patients (46 eyes) were recruited. Screening for DR and diabetic macular edema (DME) were done through two methods: (1) Clinical grading with slit lamp biomicroscopy and a 90-diopter lens, (2) Smartphone imaging with iPhone6s (Apple, Inc., Cupertino, California, USA) and a 20-diopter lens. Two independent retina specialists graded the smartphone photos for DR.

Agreement of DR and DME grading based on smartphone images and the clinical diagnosis was then assessed using the kappa statistic (simple and linear weighted). Inter-rater agreement for DR grading for the smartphone photos was also taken using the kappa statistic. Sensitivity, specificity, positive and negative predictive values for referable diabetic retinopathy were then determined.

RESULTS. Agreement between smartphone and clinical grading for DR was good ($\kappa = 0.80$, 95% confidence interval [CI], 0.66–0.95; weighted $\kappa = 0.75$, 95% CI, 0.57–0.93) and excellent for DME ($\kappa = 0.90$, 95% CI, 0.71–1.00; weighted $\kappa = 0.86$, 95% CI, 0.60–1.00). Agreement between two retina readers for DR grading with smartphone imaging was good to excellent ($\kappa = 0.80$, 95% [CI], 0.66–0.95; weighted $\kappa = 0.89$, 95% CI, 0.80–0.98). The sensitivity and specificity for referable DR, defined as at least moderate non-proliferative DR and/or DME, were 100% (95% CI, 71.5 to 100) and 91.8% (95% CI, 76.3 to 98.l), respectively.

CONCLUSIONS. Dilated smartphone fundus imaging with remote interpretation by a retina specialist is a highly sensitive and specific tool for detecting diabetic retinopathy among DM patients in the community, especially in resource-poor settings.

UK Biobank Retinal Imaging Grading: Methodology, Baseline Characteristics and Findings for Common Ocular Diseases

K. Curran[1], A. Warwick[2,3], B. Hamill[1], A.P. Khawaja[4,5], P.J. Foster[5] and T. Peto[1] on behalf of the NetwORC UK and the UKBB Eye and Vision Consortium

[1]*Centre for Public Health, Faculty of Medicine Health and Life Sciences, Queen's University Belfast, Belfast, UK*
[2]*University College London Institute of Cardiovascular Science, London, UK*
[3]*Medical Retina, Moorfields Eye Hospital NHS Foundation Trust, London, UK*
[4]*University College London Institute of Ophthalmology, London, UK*
[5]*NIHR Biomedical Research Centre, Moorfields Eye Hospital NHS Foundation Trust, and UCL Institute of Ophthalmology, London, UK*

DESIGN. The UK Biobank (UKBB) study is a large, prospective cohort study.

PURPOSE. The aims of the present study were to describe the grading methods and baseline characteristics for participants who underwent fundus imaging, and to characterise individuals with retinal features suggestive of age-related macular degeneration (AMD), glaucoma and diabetic retinopathy (DR).

METHODS. Non-mydriatic colour fundus photographs and macular optical coherence tomography (OCT) scans were acquired using a Topcon 3D OCT-1000 Mark II system (Topcon, Japan). Grading was performed by trained and certified graders and quality assured by clinicians of the Network of Ophthalmic Reading Centres UK

(NetwORC UK – Belfast, Liverpool, and Moorfields Ophthalmic Reading Centres). Study-specific grading forms were created to capture retinal features including those associated with any AMD (at least one definite drusen on either imaging modality), glaucoma (based on colour fundus photographs only; ≥0.7 cup-disc ratio, ≥0.2 cup-disc ratio difference between the eyes, abnormal disc features (notching, inferior rim thinning or both) and/or disc haemorrhage in either eye) and DR (microaneurysms with or without other characteristic features of DR). Suspected cases of these conditions were compared against the information provided by UKBB self-reported (verbal interview), linked hospital episode statistics and available primary care records.

RESULTS. Among 68,517 UKBB participants who underwent retinal imaging, the median age at imaging was 58.7 years (interquartile range 50.8–64.0), 45.7% were men and 90.6% were of white ethnicity. Altogether, 64,266 (93.8%) participants had gradable colour fundus photographs and 68,465 (99.9%) had gradable OCT scans in at least one eye. Retinal features suggestive of any AMD, glaucoma and DR were identified in 15,178 (22.2%), 2,183 (3.2%) and 265 (0.4%) participants, of whom 125 (0.8%), 218 (10.0%) and 132 (49.8%) respectively had a recorded diagnosis.

CONCLUSIONS. The outcomes of grading of baseline images represents a rich resource that will aid detailed phenotyping of UKBB subjects and yields diagnostic information not captured by self-report and healthcare data linkage. Judicious use of the image analysis information generated will accelerate research across a range of areas including genetics and artificial intelligence.

Multimodal Retinal Imaging as Biomarker for Cardiovascular Disease in Patients with Diabetes Mellitus

S. Vujosevic[1,2], F. Fantaguzzi[3], R. Salongcay[4], L. Cushley[4], M. Brambilla[5], E. Torti[6] and T. Peto[4]

[1]*Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy*
[2]*Eye Clinic, IRCCS MultiMedica, Milan, Italy*
[3]*Medical School, University of Milan, Milan, Italy*
[4]*Centre for Public Health, Queen's University Belfast, Belfast, UK*
[5]*Department of Medical Physics, University Hospital Maggiore della Carità, Novara, Italy*
[6]*Department of Electrical, Computer and Biomedical Engineering, University of Pavia, Pavia, Italy*

DESIGN. Bi-center, retrospective, observational case-series.

PURPOSE. To evaluate association between macrovascular systemic comorbidities and multimodal retinal imaging in patients with diabetes mellitus (DM) with different stages of diabetic retinopathy (DR).

METHODS. 516 eyes of 259 DM patients with systemic history and imaging were enrolled in two centers (Milano and Belfast). Ultrawidefield color fundus photos (CFP) were obtained with OPTOS California and OCT/OCT-Angiography (OCT-A) imaging with Heidelberg Spectralis. The presence of predominantly peripheral lesions (PPL) was confirmed by two independent graders as >50% of CFP lesions. OCT-A imaging (3×3 mm) was used to determine perimeter, area and circularity index of the foveal avascular zone (FAZ) and vessel density (VD); perfusion density (PD); fractal dimension (FD) on superficial (SCP), intermediate (ICP) and deep (DCP) plexuses; flow voids (FV) in the choriocapillaris (CC).

RESULTS. 81.5% of patients had DM type 2, with mean age of 67.1 ± 13.7 years. Out of 516 eyes, 108 eyes (20.9%) did not have DR, and 6 eyes were not gradable. The remaining 402 eyes were: 10.3% (53) mild non-proliferative DR (NPDR), 38.2% (197) moderate NPDR, 11.8% (61) severe NPDR, 17.6% (91) proliferative DR (PDR). PPL was present in 35.5%, associated with longer DM duration and worse DR severity ($p < 0.001$). A worse DR stage was associated with a history of stroke ($p = 0.044$). Presence of stroke was associated with: decreased FD on SCP and DCP ($p = 0.011$; $p = 0.048$), decreased VD on SCP and DCP ($p = 0.011$; $p = 0.027$), and decreased PD on SCP ($p = 0.014$). Ischemic cardiopathy was associated with decreased PD and VD on ICP ($p = 0.04$; $p = 0.05$) and increased FV on CC ($p = 0.03$). The multiple regression analysis showed that FAZ circularity and VD in SCP and ICP and PD in ICP accounted for 30% of DR severity variability.

CONCLUSIONS. OCT-A metrics show an association with the cerebrovascular and cardiovascular complications of DM, providing potentially useful biomarkers for estimating systemic vascular risk in DM patients.

Retinal Fields Nonperfusion Identified on Ultrawide Field Fluorescein Angiography in Diabetic Eyes

R. Salongcay[1,2,3], L.C. Aquino[3], C.M. Salva[3], T. Peto[1] and P.S. Silva[2,3,4,5]

[1]*Centre for Public Health, Queen's University Belfast, Belfast, UK*
[2]*Eye and Vision Institute, The Medical City, Metro Manila, Philippines*
[3]*Philippine Eye Research Institute, University of the Philippines Manila, Manila, Philippines*
[4]*Joslin Diabetes Center, Beetham Eye Institute, Boston, MA, USA*
[5]*Department of Ophthalmology, Harvard Medical School, Boston, MA, USA*

DESIGN. Cross-sectional prospective study.

PURPOSE. To identify retinal fields nonperfusion on ultrawide field (UWF) fluorescein angiography (UWF-FA) and determine its correlation to diabetic retinopathy (DR) severity on UWF photos (UWF-FP) and

macular edema on optical coherence tomography (OCT) among eyes with diabetic eye disease.

METHODS. Sixty-nine eyes from 43 patients with diabetes underwent imaging using UWF-FP, UWF-FA and macular OCT. Diabetic retinopathy (DR) severity was assessed by a masked trained grader on UWF-FP using the international DR classification. UWF-FA images were segmented into ETDRS and 5 extended peripheral (P) fields. Ischemic areas were identified on UWF-FA using Image J (Fiji) software and the nonperfusion index (NPI), cones NPI (CPI) and rods NPI (RPI) within ETDRS and per peripheral field were calculated. The presence of center-involving macular edema (ciDME) was evaluated using spectral domain OCT. Pearson correlation values (r) between UWF and OCT findings were calculated.

RESULTS. Increasing DR severity was correlated with increase in NPI: macular ($r = 0.40597$, $p = 0.0011$), within ETDRS ($r = 0.50349$, $p < 0.001$), P3 ($r = 0.37752$, $p = 0.0025$), P4 ($r = 0.43884$, $p = 0.0004$), P5 ($r = 0.57411$, $p < 0.0001$), P6 ($r = 0.47896$, $p < 0.0001$), P7 ($r = 0.55364$, $p < 0.0001$). This remained significant even after distinguishing between cones [CPI: macular ($r = 0.42368$, $p = 0.0006$), within ETDRS ($r = 0.49783$, $p < 0.0001$), P3 ($r = 0.43379$, $p = 0.0004$), P4 ($r = 0.45817$, $p = 0.0002$), P5 ($r = 0.59082$, $p < 0.0001$), P6 ($r = 0.44896$, $p = 0.0003$), P7 ($r = 0.55273$, $p < 0.0001$)] and rods [RPI: macular ($r = 0.39798$, $p = 0.0014$), within ETDRS ($r = 0.49339$, $p < 0.0001$), P3 ($r = 0.44759$, $p = 0.003$), P4 ($r = 0.45979$, p0.0002), P5 ($r = 0.59311$, $p < 0.0001$), P6 ($r = 0.43911$, $p < 0.0004$), P7 ($r = 0.55348$, $p < 0.0001$)]. The presence of ciDME on OCT is correlated with increasing NPI: macular ($r = 0.51318$, $p < 0.0001$), within ETDRS ($r = 0.53196$, $p < 0.0001$), P6 ($r = 0.44692$, $p = 0.0003$), P7 ($r = 0.42262$, p0.0006); CPI: macular ($r = 0.51924$, $p < 0.0001$), within ETDRS ($r = 0.54334$, $p < 0.0001$), P6 ($r = 0.48529$, $p < 0.0001$), P7 ($r = 0.48359$, $p < 0.0001$); and RPI: macular ($r = 0.5082$, $p < 0.0001$), within ETDRS ($r = 0.53388$, $p < 0.0001$), P6 ($r = 0.4813$, $p < 0.0001$), P7 ($r = 0.4944$, $p < 0.0001$).

CONCLUSIONS. Retinal nonperfusion is associated with DR severity and ciDME. Our findings suggest that distinguishing between cones and rods nonperfusion may be inconsequential in a generalized retinal disease such as DR. Subfield nonperfusion, especially in the nasal fields (P6 and P7), needs further investigation to elucidate the possible anatomic and functional mechanisms behind its correlation to macular edema.

Macular Vascular Density in Children and Adolescents with Type I Diabetes

M. Torm[1, 3], J. Johannesen[2, 3], J.N. Hajari[1, 3], O.N. Klefter[1] and M. Larsen[1]

[1]*Department of Ophthalmology, Rigshospitalet, København, Denmark*
[2]*Department of Paediatrics, Herlev Hospital, Herlev, Denmark*
[3]*Steno Diabetes Center Copenhagen, Herlev, Denmark*

DESIGN. Observational cross-sectional study.

PURPOSE. Retinal capillary occlusion can occasionally be demonstrated in people with diabetes in the absence of fundus photographically visible retinopathy. With optical coherence tomography angiography (OCTA) evidence of occlusion is in the form of abnormally large intercapillary spaces or loss of perfusion compared to baseline examinations. It remains to be determined how early in the course of diabetes retinal microvascular occlusion can be detected. The purpose of this report is to present OCTA data from an ongoing prospective study of capillary perfusion in children and adolescents with type I diabetes.

METHODS. This observational study has currently enrolled 52 participants with type 1 diabetes and 38 age-matched healthy control subjects. Patient age range was 6–32 (mean 15) years and diabetes duration 14.5–17.5 years ($n = 9$), 9–11.5 years ($n = 14$), 4.5–6 years ($n = 17$), and 0-5 months ($n = 12$). In healthy controls, the age range was 7–34 (mean 16) years. Exclusion criteria were significant chronic systemic disease other than diabetes and ocular disease other than diabetic retinopathy. Examinations included 3×3 mm macular OCTA scans (Topcon Triton) and fundus photography (Optos). Capillary densities of four macular ETDRS grid sectors were calculated using proprietary software (Topcon ImageNet6) (Figure 1).

RESULTS. Of 52 patients with type I diabetes, 42 had no fundus photographic retinopathy, 7 had very mild and 3 had mild non-proliferative diabetic retinopathy. Mean superficial capillary plexus density in per cent in the temporal, superior, nasal, and inferior perifoveal sectors were 47.5, 50.2, 47.1, and 50.0, respectively, in healthy controls, 47.8, 49.0, 47.2, and 49.6 in patients with diabetes duration 0-5 months, and 47.0, 49.0, 46.6, and 48.7 in patients with diabetes duration 4.5–17.5 years.

CONCLUSIONS. Preliminary cross-sectional OCTA data show a trend toward decreasing macular capillary density with increasing diabetes duration in a young type I diabetes cohort where only 1 in 5 participants had fundus photographic retinopathy. Ongoing efforts are aimed at combining structural and angiographic OCT data to enable routine mapping of occluded capillaries.

Diagnosis of Diabetic Neuropathy by Artificial Intelligence using Corneal Confocal Microscopy

Y. Meng[1], M. Ferdousi[3], I.N. Petropoulos[4], R.A. Malik[4], Y. Zhao[7], U. Alam[2,6] and Y. Zheng[1,5]

[1]*Department of Eye and Vision Science, Institute of Life Course and Medical Sciences, University of Liverpool, Liverpool, UK*

[2]Institute of Life Course and Medical Sciences and the Pain Research Institute, University of Liverpool and Liverpool University Hospital NHS Foundation Trust, Liverpool, UK
[3]Institute of Cardiovascular Science, University of Manchester and Manchester Diabetes Centre, Manchester Foundation Trust, Manchester, UK
[4]Weill Cornell Medicine – Qatar, Doha, Qatar
[5]St Paul's Eye Unit, Royal Liverpool University Hospital, Liverpool, UK
[6]Division of Endocrinology, Diabetes and Gastroenterology, University of Manchester, Manchester, UK
[7]Cixi Institute of Biomedical Engineering, Ningbo Institute of Materials Technology and Engineering, Chinese Academy of Sciences, Ningbo, China

DESIGN. Retrospective cohort study.
PURPOSE. Corneal confocal microscopy (CCM) is a non-invasive technique that images the corneal subbasal nerve plexus. CCM can detect diabetic peripheral neuropathy (DPN) based on quantification of corneal subbasal nerve plexus. There are a paucity of tools for the analysis of CCM images for the diagnosis of DPN. We proposed a deep learning algorithm for the classification of peripheral neuropathy (PN+) versus a lack of peripheral non-neuropathy (PN-).
METHODS. The algorithm was developed by adopting a residual neural network called ResNet-50 to perform the classification task. An additional dropout layer with a dropout rate of 0.6 was added to increase the algorithm's generalisability. Data augmentation techniques, such as random rotation and flip, were used during the training process to avoid overfitting problems so as to increase the algorithm's generalisability. The stochastic gradient descent with a momentum of 0.9 was adopted to optimize the algorithm. The algorithm was trained for 300 epochs with a start learning rate of 0.0001.
The algorithm was developed and evaluated on a CCM dataset of 301 patients (257 PN−, 144 PN+), utilising one image per patient. Peripheral neuropathy was defined using international criteria (Toronto Consensus criteria).Corneal images were collected using standard, validated protocols. The dataset was randomly split into training, validation and testing sets, which contains 203 (109 PN− 94 PN+), 37 (19 PN−, 18 PN+) and 61 images (29 PN−, 32 PN+), respectively. Sensitivity, specificity and area under curve (AUC) were used to measure the diagnostic performance.
RESULTS. The results on the test dataset were as follows with 95% confidence interval (CI): Specificity of 0.91 (95% CI: 0.79–1.0), Sensitivity of 0.93 (95% CI: 0.83–1.0), AUC of 0.95 (95% CI: 0.89–0.99). Gradient-weighted class activation maps were ascertained and provided the important areas for the decision-making.
CONCLUSIONS. Our algorithm demonstrates promising results for the diagnosis of peripheral neuropathy using CCM when compared to reference standard criteria. A large-scale multicenter validation study in a clinical population is required prior to its establishment in diabetes screening and diagnostic programmes.

Association of Ultrawide Field Fluorescin Angiography-Identified Retinal Non-Perfusion with Risk of Diabetic Retinopathy Worsening Over Time: Results from the DRCR Retina Network Protocol AA

P.S. Silva[1] on behalf of the DRCR Retina Network

[1]Joslin Diabetes Centre, Department of Ophthalmology, Harvard Medical School, Boston, MA, USA

DESIGN OF STUDY: Prospective multicenter longitudinal observational study.
PURPOSE. To assess whether extent and location of ultrawide field fluorescein angiography (UWF-FA) identified retinal nonperfusion (NP) and predominantly peripheral lesions (FA-PPL) are associated with diabetic retinopathy (DR) progression over time.
METHODS. A subset of eyes enrolled in a prospective multicenter longitudinal observational study conducted by DRCR Retina Network were analysed. All 508 eyes had non-proliferative DR and gradable NP on UWF-FA at baseline. Over 4 years, 200° UWF-colour images were collected annually and UWF-FA images were collected at baseline, 1 and 4 years. DR severity scale (ETDRS DRSS) level within ETDRS fields on UWF-colour images, as well as NP area (NPA), NP index (NPI), and presence of predominantly peripheral lesions on UWF-FA (FA-PPL) were graded by a centralized reading center. Treatment of DR and/or DME was at investigator discretion. The primary outcome was disease worsening over 4 years, defined as ≥2 steps DRSS worsening within ETDRS fields on UWF-colour images or receipt of treatment for DR.
RESULTS. Adjusting for baseline DR severity, the risk of DR worsening over 4 years was higher in eyes with greater overall NPI (HR for 0.1-unit increase, 1.11; 95% CI, 1.02–1.21; $p = 0.02$) and NPI within the posterior pole (1.35; 1.17–1.56; $p < 0.001$) and mid-periphery (1.08; 1.01–1.6; $p = 0.04$). In a multivariable model adjusting for baseline ETDRS DRSS and systemic risk factors including age, diabetes type, diabetes duration, haemoglobin A1c, eGFR, and albuminuria, greater NP (HR, 1.11; 95% CI, 1.02–1.22; $p = 0.02$) and presence of FA-PPL (HR, 1.89; 95% CI, 1.35–2.65; $p < 0.001$) remained associated with DR worsening independent of these factors.
CONCLUSION: This 4-year longitudinal study has demonstrated that both FA-PPL and greater baseline retinal NP on UWF-FA are associated with higher risk of DR worsening or treatment, even after adjusting for baseline ETDRS DR severity and known systemic risk factors. These associations between DR progression and retinal NP and FA-PPL support the increased use of UWF-FA to

complement color fundus photography in future efforts for DR clinical care and research.

Sub-Clinical Auditory Neural Deficits in Type 1 Diabetes Mellitus

C. J. Plack[1], A. Aljasser[2] and P. Dawes[1] on behalf of the SENSEcog Network

[1]*Manchester Centre for Audiology and Deafness, The University of Manchester, Manchester, UK*
[2]*Department of Rehabilitation Sciences, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia*

DESIGN. Although diabetes mellitus (DM) is associated with clinical hearing loss, little attention has been given to auditory neuropathic complications. Sensitive electrophysiological tests may reveal the earliest signs of damage, before elevations in the clinical audiogram are apparent.
PURPOSE. To establish early markers for diabetic neuronal damage using auditory electrophysiological measures, and determine relations to behavioural measures of function.
METHODS. Thirty young normal-hearing type 1 DM patients, and 30 age-, sex-, and audiogram-matched healthy controls. Electrophysiological measures of auditory nerve and auditory brainstem function using the click-evoked auditory brainstem response (ABR), and the sustained frequency-following response (FFR). Behavioural tests of fine temporal discrimination, and speech perception in noise.
RESULTS. No significant differences between DM patients and controls in the ABR. However, the DM group showed significantly lower FFR responses, higher discrimination thresholds, and worse speech-in-noise performance.
CONCLUSIONS. Type 1 DM is associated with degraded brainstem coding even in the absence of audiometric threshold elevation. These deficits may impact on real-world hearing ability. The FFR may provide an early marker of neural damage, before abnormalities are revealed by standard clinical tests. The SENSEcog Network will establish an approach based on combined measures of visual and auditory neural function, to allow sensitive measures of disease progression and appropriate intervention.

Interpretable Machine Learning to Identify Visual Function Measures which Best Describe Diabetic Retinopathy Stage

R. Hogg[1,2], G. Montesano[2], R. Das[1], U. Chakravarthy[1], D. Crabb[2] and D.M. Wright[1]

[1]*Centre for Public Health, Queen's University Belfast, Belfast, UK*
[2]*Division of Optometry and Vision Science, City University London, London, UK*

DESIGN. Cross-sectional cohort Study 1122 participants (2244 eyes) from the first wave of the Northern Ireland Sensory Ageing study, UK. The sample comprised 310 individuals with age-related macular degeneration, 304 individuals (584 eyes) with diabetes and the remainder with neither condition. Those with AMD were excluded from the analysis.
PURPOSE. To evaluate the extent to which visual function measures from a panel of 22 candidates analysed using interpretable machine learning techniques are able to discriminate (1) Eyes from those with diabetes but no DR from those with no diabetes (2) Eyes from those with DR from those with diabetes but no DR.
METHODS. Presence of diabetic retinopathy (DR) was determined using fundus photography, ultra-widefield imaging (Optomap) and OCT imaging.
For each task, predictor variables were age, sex and the visual function measures. Missing measurements were imputed using chained equations.
An ensemble of machine learning models was applied for each task, using the SuperLearner algorithm to find the optimum weighting of component models. Predictive performance of the ensemble was compared to standard statistical methods (multiple regression).
An interpretable machine learning approach (SHAP values) was used to identify variables with the greatest influence on the ensemble predictions, and to identify clusters of eyes where predictions were made for similar reasons.
RESULTS. Task 1: The ensemble approach correctly distinguished those with diabetes but no DR from those with no diabetes in 99% of eyes, achieving 96% sensitivity for detecting diabetes but no DR. In contrast, multiple regression correctly classified 84% of eyes but only achieved 10% sensitivity. Features that drove predictions of diabetes included below average reading speed and below average microperimetry sensitivity.
Task 2: the ensemble approach correctly distinguished those with DR from those with diabetes but no DR in 98% of eyes, achieving 99% sensitivity for detecting DR. Multiple regression correctly classified 65% of eyes and only achieved 66% sensitivity. Below average reading speed was a prominent predictor of DR.
CONCLUSIONS. Machine learning demonstrated excellent performance for both tasks. This approach shows promising potential for monitoring of diabetic retinopathy progression.

Poster Sessions

Incidence of Sight and Severe Sight Impairment Certifications from 2010 to 2020 due to Diabetic Retinopathy in England and Wales

R. Thomas[1], A. Zekite[2], R.V. North[3], R. Reynolds[4], G.S. Williams[5], D. Flanagan[2] and D.R. Owens[1]

[1]Diabetes Research Group, Swansea University Medical School, Swansea, UK
[2]Moorfields Eye Hospital Foundation Trust, London, UK
[3]School of Optometry and Vision Sciences, Cardiff University, Cardiff, UK
[4]Department of Ophthalmology, Aneurin Bevan University Health Board, Newport, UK
[5]Department of Ophthalmology, Swansea Bay University Health Board, Swansea, UK

DESIGN. Retrospective observational study.
PURPOSE. The World Health Organisation (WHO) highlighted the importance of reporting trends and progress in preventing avoidable blindness in its 2009-2013, in order to evaluate the impact of different strategies. This study aims to understand the trends in the incidence of sight (SI) and severe sight impairment (SSI) certifications over a decade prior to the Covid-19 pandemic.
METHODS. Certifications for SI and SSI in England and Wales are received by the certification's office at Moorfields Eye Hospital, London.
RESULTS. Since 2010 there has been a gradual reduction in certifications for SI and SSI in England and Wales from 72.8 to 41.3 per 100,000 people with diabetes in England and from 82.3 to 55.5 per 100,000 in Wales. However, in Wales since 2016 there has been a gradual increase from 43.5 to 55.5 per 100,000 people with diabetes, similar to that recorded in 2013–2014 at 58.5 per 100,000. This coincides with an increase in the number of certifications for SI and SSI in Wales of 15.7% in those aged 12–34 year and 8% in those 55–69 years respectively, as well as an overall increase in all cause certifications in Wales since 2015 from 40.1 to 51.8 per 100,000 people.
CONCLUSIONS. Over the last decade there has been a gradual decrease in certifications of SI and SSI due to diabetic retinopathy in both England and Wales. However, in contrast to England there has been a slight reversal in this trend in Wales since 2016 which requires further investigation.

Characterization of Two-Year Progression of Risk Phenotypes of Diabetic Retinopathy

L. Ribeiro[1,2], R. Coimbra[1], T. Santos[1], M.H. Madeira [1,2], A.R. Santos[1,2,4], C. Lobo[1,2,3] and J. Cunha-Vaz[1,2]

[1]AIBILI - Association for Innovation and Biomedical Research on Light and Image, Coimbra, Portugal
[2]Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, University of Coimbra, Coimbra, Portugal
[3]Department of Ophthalmology, Centro Hospitalar e Universitário de Coimbra (CHUC), Coimbra, Portugal
[4]Department of Orthoptics, School of Health, Polytechnic of Porto, Porto, Portugal

DESIGN. Prospective observational 2-year study.
PURPOSE. To characterize the two-years progression of two diabetic retinopathy (DR) risk phenotypes in type 2 diabetes (T2D).
METHODS. A prospective longitudinal cohort study (CORDIS, NCT03696810) was conducted with 4 visits (baseline, 6-months, one-year and two-year). Demographic and systemic data included age, sex, diabetes duration, lipidic profile and hemoglobin A1c (HbA1c). Ophthalmological examinations including visual acuity (BCVA), color fundus photography (CFP) and optical coherence tomography (OCT and OCTA), identified the presence of nonproliferative diabetic retinopathy (NPDR). Phenotype classification was performed, at 6-month visit, based on microaneurysm turnover (MAT, on CFP) and central retinal thickness (CRT, on OCT). Only risk phenotypes B (MAT < 6 and increased CRT) and C (MAT ≥ 6 with or without increased CRT) were included. ETDRS grading was performed at the baseline and last visits based on 7-fields CFP.
RESULTS. 133 T2D individuals were included in the study, 81 (60%) eyes classified as phenotype B and 52 (40%) eyes as phenotype C. Of these, 127 completed the two-year follow-up, 24 (19%) developed central-involved macular edema (CIME) and 2 clinically significant macular edema (CSME) (1.6%). In the two-year period, two-step severity progression (ETDRS) occurred only in one eye with phenotype C.
At baseline, eyes with phenotype C showed more capillary closure in the superficial capillary plexus (SCP), deep capillary plexus (DCP) and full retina (FR, $p < 0.001$) and increased FAZ area ($p < 0.001$), indicating more advanced microvascular disease and confirming the ischemia phenotype. During the two-year period both phenotypes, B and C, showed progression in GCL + IPL thinning ($p < 0.001$) and decrease in vessel density in the DCP (<0.001). When analysing the two-year progression of each phenotype, only phenotype C revealed significant decrease in BCVA ($p = 0.02$) and enlargement of the FAZ ($p = 0.03$). CSME developed only in phenotype C whereas CIME occurred in both risk phenotypes.
CONCLUSIONS. In the two-year period of follow-up both phenotypes B and C showed progression in retinal neurodegeneration associated with progression in capillary closure identified by progressive decrease in vessel density of the DCP. CIME developed in both phenotypes and CSME only in phenotype C.

Characterization of Two-Year Progression of Capillary Closure in Nonproliferative Diabetic Retinopathy

I. Marques[1,2], L. Ribeiro[1,2], S. Ferreira[1], A.R. Santos[1,2,3], T. Santos[1] and J. Cunha-Vaz [1,2]

[1]AIBILI - Association for Innovation and Biomedical Research on Light and Image, Coimbra, Portugal
[2]Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, University of Coimbra, Coimbra, Portugal
[3]Department of Orthoptics, School of Health, Polytechnic of Porto, Porto, Portugal

DESIGN. Prospective observational 2-year study.

PURPOSE. To characterize the two-year progression of capillary closure in different diabetic retinopathy (DR) risk phenotypes in type 2 diabetes (T2D).
METHODS. A prospective longitudinal cohort study (CORDIS, NCT03696810) was conducted with 4 visits (baseline, 6-months, one-year and two-year). Demographic and systemic data included age, sex, diabetes duration, lipid profile and haemoglobin A1c (HbA1c). Ophthalmological examinations including visual acuity (BCVA), colour fundus photography (CFP) and optical coherence tomography (OCT and OCTA) identified nonproliferative diabetic retinopathy (NPDR). Phenotype classification was performed, at 6-month visit, based on microaneurysm turnover (MAT, on CFP) and central retinal thickness (CRT, on OCT). Only risk phenotypes B (MAT < 6 and increased CRT) and C (MAT ≥ 6 with or without increased CRT) were included. ETDRS grading was performed at the baseline and last visit based on 7-fields CFP.
RESULTS. 133 T2D individuals were included in the study, 81 (60%) eyes classified as phenotype B and 52 (40%) eyes as phenotype C. Of these, 127 completed the two-year follow-up with 24 (19%) developing central-involved macular edema (CIME) and 2 (1.6%) clinically significant macular edema (CSME).
At baseline, eyes with phenotype C showed more capillary closure in the superficial capillary plexus (SCP), deep capillary plexus (DCP) and full retina (FR), $p < 0.001$) and increased FAZ area ($p < 0.001$), indicating more advanced ischemic disease. During the two-year follow-up period, the decrease in skeletonized vessel density indicating capillary closure, occurred mainly in the DCP in both phenotypes. Positive associations with the increased capillary closure were identified with GCL+IPL thinning (representing neurodegeneration) and decreased BCVA. Using a combination of parameters such as FAZ metrics, vessel density and degree of neurodegeneration it was possible to identify different risk profiles in eyes with the same ETDRS levels.
CONCLUSIONS. Significant progression in capillary closure was identified by a decrease in vessel density in the deep capillary plexus and increase in the FAZ area combined with progression in neurodegeneration (identifying different risk profiles in eyes with the same ETDRS grade). Eyes developing CIME had less decrease in vessel density of the SCP at baseline.

Addressing Technical Failures in a Diabetic Retinopathy Screening Programme

E. McBride[1], D. Garrahy[1], R.W.A. Acheson[1], D. Keegan[2], C. Murphy[2], S. McMahon[1] and L. O'Toole[1]

[1]*NEC Care, Cork City, Co Cork, Ireland*
[2]*Diabetic RetinaScreen, National Screening Service, Health Service Executive, Dublin, Ireland*

DESIGN. A retrospective review of the technical failure (TF) rate over a 24-month period.
PURPOSE. The consequence of a TF at diabetic retinopathy screening, is that the patient must be reassessed at a second slit-lamp examination. This is inconvenient for the patient as well as costly to the screening programme. We looked to identify why TFs occur and if by modifying our protocol they could be avoided.
METHODS. The TF rate was determined at monthly intervals over a 24 month period from February 2019 to February 2021. An analysis for the specific reasons for TF was performed at six monthly intervals. Interventions introduced during the 24 months included the use of G Phenylephrine 2.5%, the capture of 3 instead of 2 fundal images, regular camera servicing, and multidisciplinary teaching (MDT) sessions.
RESULTS. In Feb 2019 the TF rate was 14% (529/3357), in Feb 2021 it had reduced to 5% (199/4410). The most common cause of TF at each 6 monthly analysis was cataract 48% (range 40–60). The number of artefact-related TF fell from 30% to 5% over 24 months. Non-modifiable causes for TF included asteroid hyalosis, corneal scarring, and patients not being able to appropriately position at the camera.
Following the introduction of MDT and conversion to three rather than two image acquisition, the TF rate reduced from 14% in Feb 2019 to 6% a month later. The introduction of G Phenylephrine 2.5% combined with MDT resulted in a decrease in the TF rate from 7% in July 2020 to 5% two months later.
CONCLUSIONS. MDT sessions alone or combined with other interventions, resulted in a significant reduction in the TF rate. This underlines the need for ongoing educational sessions for screeners. Cataracts account for the majority of TFs in our catchment area, and despite full dilation and multiple image acquisition, most patients with cataracts still require referral to our optometric services.

Teleophthalmology Diabetic Retinopathy Screening Program in Castilla y León

I.O.B.A. Laurencio[1], M.I. Lopez[1], J.C. Pastor[1], L. Mena[1] and P. Arlanzon[1]

[1]*Reading Centre of the Eye Institute of University of Valladolid, Valladolid, Spain*

DESIGN. A prospective cohort study was conducted in the Tele-ophthalmology Diabetic Retinopathy Screening Program in Castilla y León; between January 1 to December 30 of 2021, with 45 health centers recruiting in Valladolid and Palencia and a centralized reading center grading the images.
PURPOSE. Diabetic retinopathy (DR) is a leading cause of blindness worldwide. Tele-ophthalmology based diabetic retinopathy screening programs are an important

tool in the prevention of vision loss and in our country are based within primary care. In this work a new system based on an external reading center and utilising certified technicians has been evaluated.
METHODS. Digital retinal images were captured by certified technicians using a nonmydriatic fundus camera and transferred via teleophthalmology screening program to the external reading center. Trained and certificated opticians in the reading of fundus images carried out the first reading and ophthalmologist trained in retinal disease completed the reading of those images with pathology and created the DR status reports.
RESULTS. A total of 4,464 patients were evaluated during 2021: 2,601 from East Valladolid, 634 from West Valladolid, and 1,229 from Palencia, of which 13.01% (581 of 4464) of patients were diagnosed with DR but only 1.52% (68 of 4464) had to be referred to the corresponding hospital ophthalmology services. Only seven patients were referred to the ophthalmology services for other pathologies not related to DR.
CONCLUSIONS. The Teleophthalmology Diabetic Retinopathy Screening Program is an effective way to diagnose DR so patients can be referred and treated appropriately.

Evaluating the Efficiency of the Irish National Diabetic Retinal Screening Service's Treatment Pathway

I. Brennan[1,2] and D.J. Keegan[1,2]

[1]*Mater Vision Institute, Dublin, Ireland*
[2]*Diabetic RetinaScreen, National Screening Service, Health Service Executive, Dublin, Ireland*

DESIGN. Diabetic retinal screening is available to all people with diabetes in the Republic of Ireland over the age of 12 through a national screening service (Diabetic RetinaScreen). Quality assurance (QA) is a vital part of managing the quality of service available through Diabetic RetinaScreen. One key focus of the system's QA standards is the timeliness of both clinical review and treatment of disease identified through screening.
PURPOSE. To assess the timeliness of clinical review and treatment of patients referred to ophthalmology through Diabetic RetinaScreen from 2018–2020.
METHODS. Information was collected and organised from the Diabetic RetinaScreen's centralised electronic record keeping software, OptoMize (NEC, Japan). 20213 referrals were sent from screening for ophthalmology review from 2018–2020, of which 13156 had referable retinopathy (2802 urgent, 10354 routine). Of these, 1224 patients were listed for treatment (636 urgent, 588 routine). Intervals between screening, referral, consultation and treatment were recorded.
RESULTS. From 2018–2020, 97.67% of referrals from screening to ophthalmology were sent within the target timeframe (minimum 95% within ≤12 business days). Targets for patient review by an ophthalmologist, however were missed for 52.03% of urgent referrals (target 60% patients ≤ 12 business days / 95% patients≤ 24 business days) and 64.84% of routine referrals (target 70% patients ≤ 78 business days / 95% patients ≤ 108 business days) Additionally, targets for timely treatment following patient listing were also missed for 63.21% of urgent cases (target 90% patients ≤ 12 business days) and 60.71% of routine cases (target 70% patients ≤ 60 business days).
CONCLUSIONS. The treatment pathway set out by Diabetic RetinaScreen did not meet the quality standards the service had set itself. While treatment delays have been exacerbated by the COVID-19 pandemic, root-cause analysis of system delays pre-pandemic remains essential in understanding the shortcomings of this treatment pathway. Through understanding the causes of system delays, we can hopefully improve efficiency of this pathway as the system recovers.

Five-year Outcomes of Diabetic Eye Screening in Patients Aged 85 Years

A. Hamid[1,2], H.W. Wharton[1,2] and S.J. Jacob[1,2,3]

[1]*Solihull and Black Country Diabetic Eye Screening Programme, Birmingham, UK*
[2]*University Hospitals Birmingham, Birmingham, UK*
[3]*Aston University, Birmingham, UK*

DESIGN. Retrospective audit.
PURPOSE. To assess the incidence of referable diabetic retinopathy (DR) and treatment needed in patients aged 85 years over a follow up period of five years to determine whether screening interval can be extended safely in this age group.
METHODS. Patients who attended screening aged 85 years during April 2014-March 2015 were included. Screening results at baseline and over the next five years were analysed along with the patient demographics. Referable DR was classed as maculopathy, pre-proliferative or proliferative changes.
RESULTS. 1105 patients aged 85 years were included. Average age at their first ever screen was 73 years and the majority of the patients were Caucasian (48.8%). At 85 years (baseline screen of 1105 patients) 14 (1.3%) were referred to HES and 3 required treatment. At year 1 follow up (FU) 900 patients were screened, 2 (0.2%) were referred to HES with 0 requiring treatment. At year 2 FU, 833 were screened, 6 (0.5%) were referred to HES and 0 received treatment. At year 3 FU, 679 patients were screened, 1 (0.1%) was referred to HES and 0 had treatment. At year 4 FU, 582 were screened, 4(0.4%)

were referred to HES and 1 received treatment. At year 5 FU, 217 were screened, 1 (0.1%) was referred to HES and 0 received treatment.
In total 28 (2.53%) were referred to HES and 4 (0.4%) received treatment. Over the course of the FU, 623 (56.4%) died.
CONCLUSIONS. This study showed that only a small proportion of patients were referred to HES for DR and an even smaller proportion of patients required any treatment for DR in the eye clinics over the five years that they were followed up. These data match the five-year outcomes of screening noted in the 80-year-old cohort studied by us earlier confirming that this age group of 80–90 years has a very low risk of sight loss from DR. Screening attendance rates decreased due to death, illness or cognitive impairment making them no longer suitable for screening. It is possible to consider increasing screening interval to 5 years in this cohort of patients with optional screening in between, considering the results of the data from this study.

Assessment of Proliferative Diabetic Retinopathy Biomarkers in Vitreous Fluid

V.A. Biletskaya[1,2], D.V. Lipatov[1,2], V.K. Surguch[2] and M.A. Frolov[1]

[1]*RUDN University, Moscow, Russia*
[2]*The National Medical Research Center for Endocrinology, Moscow, Russia*

DESIGN. Case control.
PURPOSE. To study diagnostic significance of biomarkers of proliferative process: VEGF (vascular endothelial growth factor), TGF-β2 (transforming growth factor beta), HGF (hepatocyte growth factor), IL-6 (interleukin 6), IL-8 (interleukin 8).
METHODS. The study has been conducted at The National Medical Research Center for Endocrinology. Samples of vitreous were obtained in 39 patients (39 eyes) during elective vitreoretinal surgery. Patients were divided into 3 groups - (1) 16 patients with type II DM and proliferative diabetic retinopathy (PDR) complicated by significant fibrovascular proliferation, (2) 16 eyes with type II DM, less severe proliferative diabetic retinopathy and vitreous haemorrhage and (3) 7 patients without DM with rhegmatogenous retinal detachment or macular hole. Concentration of cytokines was measured using multiplex analysis with «Bio–Plex 200" analyzer («Bio–Rad»). Quantitative indicators having a normal distribution were described using arithmetic averages (M).
RESULTS. Concentration of VEGF in the group (1) (M/pg/ml) 17,25; (2) – 35,70;(3) – 0,40; IL-8 (M/pg/ml) respectively (1) 17,06; (2) – 27,85; (3) – 0,00; IL-6(M/pg/ml) (1) 18,08; (2) – 6,08; (3) – 1,26; TGF-β2 (M/pg/ml) (1) 380,70; (2) - 354,65; (3) - 75,16; HGF (M/pg/ml) (1) 26,191,65; (2) – 21528,35; (3) - 1948,65. The role of VEGF in pathogenesis of PDR is established and well-studied. Through its vascular permeability effect VEGF leads to increased fibrin deposition that can be evidenced by increased fibrin concentration. Compared to group (3) without DM both groups with DM and PDR had significantly higher concentration of VEGF.
It was shown that prolonged presence of IL-8 in circulation in response to inflammatory stimulus may case tissue damage of various degree. According to our results concentration of IL-8 in 1.6 times higher in the group with DM and less severe PDR. Induced and supported by IL-8 chronic leukocytic inflammation of vascular wall finally causes capillary occlusion and ischemia of retina.
CONCLUSIONS. Our results suggest that VEGF and IL-8 can be regarded as markers of prolonged course of proliferative diabetic retinopathy.

Presence and Development of Diabetic Retinopathy in 153,238 Patients with Type 2 Diabetes in the Danish Registry of Diabetic Retinopathy

J. Grauslund[1,2,3], F.N. Pedersen[1,2], N. Andersen[4], J. Andresen[4], S. Dinesen[1,2,3], A.S. Thykjær[1,2,3] and L. Stokholm[5]

[1]*Department of Ophthalmology, Odense University Hospital, Odense, Denmark*
[2]*Department of Clinical Research, University of Southern Denmark, Odense, Denmark*
[3]*Steno Diabetes Center Odense, Odense University Hospital, Odense, Denmark*
[4]*Organization of Danish Practicing Ophthalmologists, Copenhagen, Denmark*
[5]*OPEN – Open Patient data Explorative Network, Odense University Hospital & University of Southern Denmark, Odense, Denmark*

DESIGN. Registry-based study.
PURPOSE. To evaluate the prevalence and incidence of diabetic retinopathy (DR) along with associated markers in patients with type 2 diabetes in the Danish DR-screening program.
METHODS. We included all persons with type 2 diabetes in the Danish Registry of Diabetic Retinopathy, who had attended at least one episode of DR-screening in 2013-2018. DR was classified as levels 0-4 indicating increasing severity. Data were linked with various national health registries to retrieve information on diabetes duration, marital status, comorbidity, and systemic medication.
RESULTS. Among 153,238 persons with type 2 diabetes, median age and duration of diabetes were 66.9 and 5.3 years, and 56.4% were males. Prevalence and 5-year incidences of DR, 2-step-or-more progression of DR and progression to proliferative DR (PDR) were 8.8%, 3.8%, 0.7% and 0.2%, respectively. In multivariable models, leading markers of incident DR and progression to PDR were duration of diabetes (HR 1.98, 95% CI 1.87-2.09; HR 2.89, 95% CI 2.34-3.58 per ten years of duration) and use of insulin (HR 1.88, 95% CI 1.76–2.01; HR 2.40, 95% CI

1.84–3.13), while use of cholesterol lowering medicine was a protecting marker (HR 0.87, 95% CI 0.81–0.93; HR 0.70, 95% CI 0.52–0.93). From 2013 to 2015, 3-year incidence rates of PDR decreased from 1.22 to 0.45 events per 1,000 person-years.
CONCLUSIONS. In an entire nation of individuals with type 2 diabetes attending DR-screening, we identified duration of diabetes and use of insulin as the most important predictor for development of DR, while cholesterol lowering medicine was a protective factor.

The effect of Deferred Injection Treatment due to COVID-19 on Disease Activity in Patients with Diabetic Macular Oedema

G. Weisner[1], H.C. Cook[1] and L.D. Downey[1]

[1]*Hull Royal Eye Hospital, Hull Royal Infirmary, Hull University Teaching Hospitals NHS Trust, Hull, UK*

DESIGN. Retrospective cohort.
PURPOSE. Injections administered into the eye are currently the most effective treatment for diabetic macular oedema (DMO), blocking the action of a chemical called vascular endothelial growth factor (VEGF) and reducing swelling of the macula. In 2020, injections where deferred, following the Royal College of Ophthalmologists (RCOphth) pandemic protocol. The purpose of this study was to ascertain any change in disease activity in patients with ongoing injections for DMO, who had their injections deferred due to COVID-19. Any characteristics predicting the diagnosis could help prioritise patients, when delays occur in non-COVID times.
METHODS. This retrospective study included 125 eyes receiving ongoing injection treatment for DMO at 18.03.20, who had their injections deferred due to COVID-19 for any period onwards. Patient demographic, period of deferral and last planned injection interval prior to deferral were analysed. Once reassessed, the study evaluated if additional injections were necessary, disease stability, decreasing vision by 15 letters or more and necessary additional treatment. Diabetic retinopathy (DR) status was assessed before and after deferral.
RESULTS. The mean durations of deferral and planned last injection interval were 96 days and 7.8 weeks, respectively. Once reassessed, 36 eyes did not require further injections, 52 eyes showed stable disease, vision had decreased by 15 letters or more in 10 eyes and 8 eyes required additional treatment. Overall, patients with stable disease and no longer requiring injections (32%) had a longer mean duration of deferral (123 days) and injections further apart prior to deferral (9.7 weeks). 50% of patients showed no change, 24% improved and 18% worsening DR.
CONCLUSIONS. This study found that 1/3 of DMO-patients could discontinue injections after a period of deferral without any harm in the immediate follow-up period and without need to restart treatment. In addition, it supports the strategy to deprioritise patients where an 8-week interval or more is planned, when injection capacity is compromised. However, deferral of DMO-patients receiving active injections can cause harm in a minority of patients and should not be attempted for future COVID-19 pandemic surges.

Predictive Factors Associated with Anatomical and Functional Outcomes Following Panretinal Photocoagulation in People with Proliferative Diabetic Retinopathy

J. Perais[1, 2], P.M. McCullough[1], G.A. McLaughlin[1], E.W.J. Pritchard[2], G. Virgili[2] and N. Lois[1, 2]

[1]*The Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK*
[2]*The Ophthalmology Department, Belfast Health and Social Care Trust, Belfast, UK*

DESIGN. Retrospective clinical study.
PURPOSE. To determine the effects of baseline characteristics and laser type on clinical outcomes in people with proliferative diabetic retinopathy (PDR) undergoing panretinal photocoagulation (PRP).
METHODS. Medical records of all consecutive patients with PDR naïve to PRP, identified using an electronic database, evaluated at the Macula Clinic, Belfast Health and Social Care Trust, receiving their first PRP between 1st January 2016 and 30th June 2017, and followed for a minimum of 6 months following stabilisation of PDR, were retrospectively reviewed. Outcomes included time to stabilisation following PRP, progression of PDR, and mean change in best-corrected visual acuity from baseline to last follow-up. Cox regression was used to estimate hazard ratios for the effect of baseline characteristics and type of laser on outcomes following treatment.
RESULTS. One hundred and fourteen patients (135 eyes) with a mean age of 57.6 (SD: 13.1) years were included, 67% males. People receiving pattern or mixed laser had a statistically significantly delayed stabilisation (HR: 0.54, $p = 0.004$; and HR: 0.41, $p = 0.001$, respectively) and increased risk of progression (HR: 1.83, $p = 0.028$; and HR: 2.04, $p = 0.018$, respectively) when compared to those receiving standard laser. Among other potential predictors in multivariable regression analysis, only vitreous haemorrhage and fibrosis or traction at baseline increased risk of progression (HR: 1.70, $p = 0.017$; and HR: 4.14, $p < 0.001$ respectively). Baseline characteristics and type of laser had no statistically significant effect on vision.
CONCLUSIONS. Multi-spot pattern laser may not be as effective as standard laser in treatment of PDR, being

associated with increased number of laser burns, treatment sessions, time to stabilisation and risk of complications, many of which required treatment. This has important implications for patients and healthcare systems, including increased utilisation of resources and associated costs, particularly salient in the current climate when services are under pressure to meet demands. A methodologically sounded and appropriately powered RCT comparing acceptability, clinical and cost effectiveness is necessary to determine the real benefits, if any, pattern laser provides in treatment of PDR.

Examining whether Growth Differentiation Factor-15 is Altered in the Early Stages of Diabetic Retinopathy in Individuals with Type 2 Diabetes

K. Gooding[1,2], F. Casanova[1], C. Ball[2], C. Anning[2], N. Pamphilon[2], R. Ling[3] and A.C. Shore[1,2]

[1]*Diabetes and Vascular Medicine, University of Exeter Medical School, Exeter, UK*
[2]*NIHR Exeter Clinical Research Facility, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK*
[3]*West of England Eye Unit, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK*

DESIGN. Cross-sectional study.
PURPOSE. Circulating levels of growth differentiation factor-15 (GDF-15), a member of the transforming growth factor-β superfamily, are associated with insulin resistance, incidence of diabetes, kidney function and metformin use. Furthermore, it has been shown to be altered with increasing diabetic retinopathy severity in Asian individuals with type 2 diabetes. The aim of this research is to examine whether GDF-15 is altered in early stages of diabetic retinopathy in type 2 diabetes.
METHODS. Participants with type 2 diabetes and either no or early diabetic retinopathy with GDF-15 measurements were selected from the Exeter SUMMIT Diabetic Retinopathy cohort. Diabetic retinopathy was graded from fundus photography in line with the English Diabetic Retinopathy Grading scheme. Early retinopathy was defined as background retinopathy (R1). Circulating GDF-15 was analysed by the Proximity Extension Assay (PEA) technique using the Proseek Multiplex CVD96 × 96 reagents kit (Olink Bioscience, Uppsala), data are presented as arbitrary units.
RESULTS. 214 Caucasian individuals with type 2 diabetes were selected, 117 and 97 in the no DR and early DR groups, respectively. There was no difference in age, HbA1c, estimated glomerular filtration rate (eGFR) between groups. Duration of diabetes was longer and urinary albumin-creatinine ratio (ACR) higher in the early DR group compared to the no DR Group. 71% and 81% of the no DR and early DR group were taking metformin, respectively. GDF-15 levels were significantly higher in the early DR compared to no DR group (No DR: mean (SD): 9.98(0.73) vs Early DR 10.33(0.81), $p < 0.001$ t-test). The difference in GDF-15 remained when adjusted for age, eGFR, HbA1c, ACR, duration of diabetes and presence of cardiovascular disease (unstandardised beta (standard error): 0.205(0.101), standardised beta: 0.130, $p = 0.043$), but was lost when further adjusted for metformin use (0.162 (0.096), 0.103, $p = 0.092$).
CONCLUSIONS. Circulating GDF-15 levels are increased in early DR in individuals with type 2 diabetes, however, this increase, at least in part, may be explained by the higher proportion of patients on metformin in the early DR group compared to the no DR group.

Integrating Diabetic Eye Screening into Regional Haemodialysis Units, Northern Ireland

L. Cushley[1,2], N.B. Quinn[1], P. Blows[1], E. McKeever[3], NI Haemodialysis Units and T. Peto[1,2]

[1]*Centre for Public Health, Queen's University Belfast, Belfast, Northern Ireland, UK*
[2]*Belfast Health and Social Care Trust, Belfast, Northern Ireland, UK*
[3]*South Eastern Health and Social Care Trust, Belfast, UK*

DESIGN. Cohort Study
PURPOSE. To determine attendance at the Diabetic Eye Screening Programme in Northern Ireland, diabetic retinopathy severity and use of handheld retinal imaging in people with diabetes attending haemodialysis units in Northern Ireland.
METHODS. All patients with diabetes attending haemodialysis clinics regionally were offered diabetic eye screening in their respective regional haemodialysis renal clinics. Macula centred and disc centred fundus images were taken using both a conventional non-mydriatic fundus camera and a handheld fundus camera. All images were then graded by trained graders and ophthalmic professionals in the Diabetic Eye Screening Programme.
RESULTS. All eligible 149 people were offered a Diabetic Eye Screening Programme appointment. Of these, 132 attended, 33.8% of whom have not been seen in >3 years and 15% had never attended Diabetic Eye Screening Programme in Northern Ireland despite multiple previous appointment offers. Our results show that approximately 24% had STDR with 12.9% requiring urgent referral to hospital eye services which is much higher than the national average of 0.4%.
CONCLUSIONS. Those on haemodialysis are at high risk for sight threatening diabetic retinopathy, implementing Diabetic Eye Screening Programme in Northern Ireland in haemodialysis clinics enables timely diagnosis and referral.

Non-Attendance Rates of Patients Attending Treatment Centres for Diabetic Retinopathy in Ireland

S. Kelly[1,2], C. Murphy[2], H. Kavanagh[2], P. Fitzpatrick[3,4], T. Mooney[4], P. Kearney[5] and D.J. Keegan[1,2,3]

[1]*Mater Retina Research Group, Mater Misericordiae University Hospital, Dublin, Ireland*
[2]*Diabetic RetinaScreen, National Screening Service, Health Service Executive, Cork, Ireland*
[3]*Programme Evaluation Unit, National Screening Service, Health Service Executive, Cork, Ireland*
[4]*School of Public Health, Physiotherapy & Sports Science, University College Dublin, Dublin, Ireland*
[5]*Department of Epidemiology, University College Cork, Cork, Ireland*

DESIGN. Retrospective cohort study
PURPOSE. The purpose of this study was to determine the patient and clinic level factors that are associated with non-attendance among patients attending treatment centres for diabetic retinopathy. A secondary aim was to understand the transition from treatment centres back to community screening after discharge.
METHODS. We modelled subsets of factors known to be associated with screening level non-attendance and determined if they are relevant for an analysis of treatment centres and discharges.
RESULTS. The overall rate of non-attendance within the treatment centres was 0.13 with the highest rates of non-attendance (0.21) found in patients who were referred for non-diabetic eye disease (NDED). Morning appointments had lower rates of non-attendance when compared to afternoon appointments (OR: 0.55). One in four discharges from the treatment centres were for repeated non-attendance. Although patients with NDED had the highest rates of non-attendance, they had the lowest risk of presenting to the screening service with a worse grade after discharge.
CONCLUSIONS. This study is the first to explore the non-attendance rates of patients attending treatment centres for DR on a national scale. Several factors that are linked with higher rates of non-attendance have been identified. Of patients who are discharged back to the screening programme, most are not at risk of worsening DR grades, although patients with active proliferative retinopathy are an exception. Future work should aim to increase the retention rate of this subpopulation.

Evaluation of Efficiency of the Treatment Pathway of Irish National Diabetic Retinal Screening Service

I. Brennan[1] and D.J. Keegan[1, 2]

[1]*Mater Vision Institute, Mater Misericordiae University Hospital, Dublin, Ireland*
[2]*Diabetic RetinaScreen, National Screening Service, Health Service Executive, Cork, Ireland*

DESIGN. National diabetic retinal screening is available to all people with diabetes in the Republic of Ireland over the age of 12 through Diabetic RetinaScreen. Quality assurance (QA) is a vital part of managing the quality of service available through Diabetic RetinaScreen. One key focus of the system's QA standards is the timeliness of both review and treatment through the treatment pathway.
PURPOSE. To assess the timeliness of clinical review and treatment of patients referred to ophthalmology through Diabetic RetinaScreen from 2018–2020
METHODS. Information was collected and organised from the Diabetic RetinaScreen's centralised electronic record keeping software, OptoMize (NEC, Japan). 20213 referrals were sent from screening for ophthalmology review from 2018–2020, of which 13156 had referrable retinopathy (2802 urgent, 10354 routine). Of these, 1224 patients were listed for treatment (636 urgent, 588 routine). Intervals between screening, referral, consultation and treatment were recorded.
RESULTS. During the study period, 97.67% of referrals from screening to ophthalmology were sent within target (minimum 95% within $\leq$12 business days). Targets for patient review by an ophthalmologist, however, were achieved in 52.03% of urgent referrals (target $\leq$ 24 business days) and for 64.84% of routine referrals (target $\leq$ 108 business days). Target timelines for treatment following patient listing were achieved for 63.21% of urgent cases (target $\leq$12 business days) and 60.71% of routine cases (target $\leq$ 60 business days).
CONCLUSIONS. The treatment pathway via Diabetic RetinaScreen faces numerous challenges to expediting patient consultation and intervention evidenced by its difficulty achieving the service's high performance targets. These challenges include infrastructural, staffing and attendance issues, along with unforeseen program roadblocks such as the COVID19 pandemic. Despite this, patient treatment times have improved year-on-year and are trending towards improvement. Root-cause analysis of treatment roadblocks pre-pandemic is warranted to better understand how to optimise treatment pathways going forward.

Evaluation of 2-hydrazino-4,6-dimethylpyrimidine (2-HDP) as a Therapeutic for the Early-Stage Treatment of Diabetic Retinopathy

J. Augustine[1], E.P. Troendle[2], T. Friedel[1], E.M. Byrne[1], P. Canning[1], A.W. Stitt[1] and T.M. Curtis[1]

[1]Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK
[2]Department of Chemistry, King's College London, London, UK

DESIGN. In vivo pre-clinical investigation of 2-HDP as a novel therapeutic for the neurovascular pathology in diabetic retinopathy.
PURPOSE. Diabetic retinopathy (DR) is a common neurovascular complication of diabetes. Retinal accumulation of the acrolein-derived advanced lipoxidation end-product, FDP-lysine (Nε-(3-formyl-3,4-dehydropiperidino-lysine), has been implicated in the pathogenesis of this condition. We have identified a new drug called 2-HDP that is effective in scavenging acrolein and preventing retinal FDP-lysine accumulation during diabetes. The aim of this study was to determine whether 2-HDP can protect against neurovascular dysfunction during diabetes.
METHODS. Male Sprague-Dawley rats were divided into three groups: (1) non-diabetic; (2) streptozotocin-induced diabetic; and (3) diabetic treated with 2-HDP administrated in their drinking water. In vivo analysis of blood pressure, body weights, water intake, HbA1c and electroretinography (ERG) were measured at 1-,3- and 6-months after diabetes induction. Immunolabelling, western blotting, cytokine arrays and the Evan's blue dye assay were carried out to study the vascular, neuronal, and glial components of the retina. Molecular Dynamics (MD) simulations were performed to investigate 2-HDP drug permeation across cellular membranes.
RESULTS. ERG a- and b-wave amplitudes were significantly reduced in diabetic controls after 3- and 6-months of diabetes and these changes were completely prevented by treatment with 2-HDP ($p < 0.01$). This drug also prevented retinal FDP-lysine accumulation, the activation of Müller cells and microglia, and neuro and vasodegenerative changes in the diabetic retina ($p < 0.05$). MD simulations have revealed that most 2-HDP molecules are protonated and do not readily cross cell membranes.
CONCLUSIONS. Our studies provide strong evidence for a key role of acrolein and FDP-lysine in the development of the neurovascular lesions associated with DR.

Unravelling the Anti-Oxidative Effects of a Bioactive Marine Molecule during Neuroinflammation Associated with Diabetic Retinopathy

A.I. Arroba[1,2], F.C.C. Cano-Cano[1], F.M.L. Martín-Loro[1], L.G.J. Gómez-Jaramillo[1], M.C.G.M. González-Montelongo[1], E.Z.M. Zubía[3] and M.A.D. Aguilar-Diosdado[1,2]

[1]Biomedical Research and Innovation Institute of Cadiz (INiBICA), Cadiz, Spain
[2]Departamento Endocrinología y Nutrición, Hospital Universitario Puerta del Mar, Cadiz, Spain
[3]Departamento de Química Orgánica, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Cadiz, Spain

DESIGN. Immunomodulatory effects of the 3-arilftalidas bioactive compound on microglia (Bv.2), macrophage cells (Raw264.7) and retinal explants from BB rat (T1DM animal model) and WT rats are to be determined. The induction of anti-oxidative response in both immune systems by 3-arilftalidas were evaluated and the crosstalk in the anti-oxidative and immune response detected
PURPOSE. The aim of this work was to investigate the effects of the 3-arilftalidas on induction of anti-oxidative response and its association to immunomodulation of the specific immune retinal system in DR progression.
METHODS. Bv.2 and Raw264.7 cells were stimulated with lypopolysaccharide (LPS), as an inflammatory stimulus with or without arilftalidas. The effects of both conditioned medium (CM) from Raw264.7 and Bv.2 cells on retinal explants were analysed in order to determine the induction of anti-oxidant response and its contribution in DR progression and immune-modulation. Retinal explants from BB rat, at 7 weeks was cultured in the presence or absence of 3-arilftalidas, and retinal explants from WT rat with both CM from Bv.2 or Raw264.7 cells. Anti-oxidant response and pro/anti-inflammatory and signalling pathways were analysed by qPCR, Western blotting and immunofluorescence.
RESULTS. In a prediabetes status (normo-glycaemia) of T1DM animal model, we found retinal inflammatory events that precede hyperglycaemia detection. Bv.2 microglial, Raw264.7 macrophage cells cultured under inflammatory environment and retinal explants from BB rats treated with 3-arilftalidas modulates the expression of oxidative pathway, such as Catalase, SOD1, Keap1, Nrf-2 and ROS levels. In retinal explants from BB rats normoglycemic, the activation of the anti-oxidative response is induced by 3-arilftalidas is able to reduce the pro-inflammatory response.
CONCLUSIONS. The oxidative processes that precede to neuroinflammation in DR is able to be modulated by 3-arilftalidas, which exerts different beneficial effects (anti-oxidative and anti-inflammatory) effects on retinal and systemic immune cells that and could be an effective alternative for DR treatment and/or prevention.

Intravitreal AAV2.COMP-Ang1 Prevents Local Capillary Blood Flow Changes in the Diabetic Mouse Retina

P. Barabas[1], E. Troendle[1,2], T. Friedel[1], A.W. Stitt[1], B.K. Ambati[3], P.M. Cummins[4,5] and T.M. Curtis[1]

[1]Wellcome-Wolfson Institute for Experimental Medicine, Queens' University Belfast, Belfast, Northern Ireland, UK
[2]Department of Chemistry, King's College London, London, UK
[3]Knight Campus, University of Oregon, Eugene, OR, USA
[4]School of Biotechnology, Dublin City University, Dublin, Ireland
[5]National Institute for Cellular Biotechnology, Dublin City University, Dublin, Ireland

DESIGN. Preclinical animal model (STZ)
PURPOSE. Diabetic retinopathy (DR) is characterized by dysfunctional blood flow regulation, which contributes to the pathogenesis of this disease. The aim of this study was to assess whether gene therapeutic delivery of angiopoietin-1 (Ang1) combined with the short coiled-coil domain of cartilage oligomeric matrix protein (AAV2.COMP-Ang1) is capable of normalizing capillary blood flow in the diabetic mouse retina.
METHODS. Streptozotocin (STZ)-induced diabetic mice of 4-6 weeks disease duration were intravitreally injected with PBS, control AAV2.GFP or AAV2.COMP-Ang1. Age-matched non-diabetic mice received PBS only. One month after vector injection, visual performance was assessed by OptoMotry and retinal capillary blood flow maps were created using a Micron IV system coupled with fluorescent microbead and fluorescein angiogram-based methods. Retina wholemounts were immunostained to determine the spatial distribution of the transgene signal.
RESULTS. Transgene expression was detectable in retinal ganglion, amacrine and Müller cells. Visual performance and nasal blood flow speeds were decreased in diabetic mice injected with PBS or AAV2.GFP compared with non-diabetic controls. Intravitreal injection of AAV2.COMP-Ang1 returned visual performance and nasal blood flow speeds back to control levels.
CONCLUSIONS. Our preclinical findings suggest that focal functional changes of the capillary bed occur in STZ-induced diabetic mice and that modulation of the Ang1-signaling pathway could represent a viable therapeutic strategy to normalize capillary blood flow during early diabetes.

Transcriptomic Analysis of Barrier Gene Expression in Human Retinal Microvascular Endothelial Cells(HRMECS) Exposed to Hyperglycaemia and VEGF

S. Tarban[1], J. Augustine [1], D.A. Simpson[1] and T.M. Curtis[1]

[1]*Wellcome–Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, Northern Ireland, UK*

DESIGN. HRMECs were used for RNA-seq and bioinformatic approaches.
PURPOSE. Clinical evidence suggests that diabetic macular oedema occurs at least in part due to breakdown of the inner blood retinal barrier (iBRB). Presently, however, our understanding of the molecular composition of the iBRB and how this is altered during diabetes remains incomplete. In the current study we have begun to address this issue by using bulk RNAseq to analyse the barrier genes expressed in HRMECs under conditions mimicking key aspects of the diabetic milieu.
METHODS. HRMECS were maintained under normoglycaemic (5 mM D-glucose) or hyperglycaemic (25 mM D-glucose) conditions for 4 weeks and then either treated or not with VEGF165(25 ng/mL) for 24 h. RNA was extracted, Libraries prepared using a TruSeq kit(Illumina) and sequencing performed on a NextSeq system(Illumina). Reads were aligned to the human genome with Star aligner and a gene count matrix generated. Cytoscape and GENEmania were used to create an in-silico model of the iBRB and changes in barrier gene expression in the presence of hyperglycaemia, VEGF or both were determined using Limma voom. For barrier genes that were significantly downregulated in hyperglycaemia, CiiiDER software was used to identify over-represented transcription factor (TF) binding sites, providing a basis to better understand how expression of these genes is altered in high glucose.
RESULTS. Average gene expression levels in normoglycaemic cells were used to build a detailed in silico model of the junctional and structural genes involved in forming the human retinal microvascular barrier. Treatment of HRMECs with hyperglycaemia caused significant downregulation of numerous junctional genes including CLDN1 and 14, CDH5, 6 and 11, CADM1 and FLRT3. In contrast, VEGF caused relatively minor changes in barrier gene expression, both in the absence and presence of hyperglycaemia. Several over-represented TF-binding sites were found in barrier genes downregulated in hyperglycaemia, including TCF12, ZNF317, FOXC2, TCFL5, REL, THAP1 and ZBTB12.
CONCLUSIONS. Hyperglycaemia, but not VEGF, causes extensive downregulation of barrier gene expression in HRMECs. These effects could contribute to iBRB breakdown during diabetes, with certain TFs involved in mediating the hyperglycaemia-induced downregulation of multiple barrier genes.

Targeting TRP Channels for the Restoration of Microvascular Barrier Function in Diabetic Macular Oedema

A. Rollo[1], S. Tarban[1], P. Barabas[1], J. Augustine[1], D.A. Simpson[1] and T.M. Curtis[1]

[1]*Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, Northern Ireland, UK*

DESIGN. High-throughput Fura2 Ca2+ signalling assays were performed in vitro using primary human retinal microvascular endothelial cells (HRMECs).
PURPOSE. Retinal microvascular leakage is a major cause of vision loss in diabetic macular oedema. Recent studies have shown that TRP channel induced Ca2+ influx in endothelial cells plays a key role in the regulation of vascular permeability. Since several endogenous TRP

channel activators are known to be upregulated in the diabetic eye, our aim was to determine whether HRMEC TRP channels might contribute to the disruption of retinal endothelial barrier function during diabetes.
METHODS. RT-PCR and analysis of RNA-sequencing data were utilised to determine TRP channel expression profiles in HRMECs. Fura2 Ca2+ assays were utilised to characterise [Ca2+]I responses to TRP activators upregulated in the diabetic eye. Routes of Ca2+ mobilisation were determined by performing these assays in the presence of selective TRP channel antagonists. Further assays were performed under high D-glucose (30 mM), using L-glucose as an osmotic control, to determine how the upregulated activators mobilise [Ca2+]i in diabetic conditions.
RESULTS. TRPV1,2, and 4, TRPC1,4, and 5, and TRPM7 were found to be expressed at the mRNA level in HRMECs. Out of seventeen upregulated TRP activators screened, histamine, thrombin, lysophosphatidylcholine (LPC) and 4-oxo-nonenal (4-ONE) gave rise to the most robust [Ca2+]I responses, with EC50 values in the nanomolar or low micromolar range. [Ca2+]i responses to these activators were attenuated by TRPV2, TRPV4, and TRPM7 antagonists, to varying degrees. In HRMECs cultured under high D-glucose, the EC50 value for thrombin underwent a large leftward shift when compared with control cells.
CONCLUSIONS. HRMEC TRPV2, TRPV4, and TRPM7 channels are activated by compounds upregulated in the diabetic eye. Under hyperglycaemic conditions, HRMECs display an increased sensitivity to thrombin. Investigation into how activation of these channels affects retinal microvascular permeability during diabetes is ongoing.

Gene Therapeutic Enhancement of Acrolein Detoxification Mechanisms for the Treatment of Diabetic Retinopathy(DR)

B.M. Karan[1], J. Augustine[1], P. Barabas[1], M. Chen[1], A.W. Stitt[1] and T.M. Curtis[1]

[1]*Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, Northern Ireland, UK*

DESIGN. In vitro investigation of ALDH1A1 gene therapy as an effective treatment for DR.
PURPOSE. Acrolein (ACR) is a toxic lipid aldehyde which can lead to cellular dysfunction and death through the formation of FDP-lysine protein adducts. Studies from our lab have shown that increased FDP-lysine levels occur in Müller cells during experimental diabetes, and that this adduct contributes to the dysfunction of these cells during DR. Aldehyde dehydrogenase 1A1 (ALDH1A1) represents one of the major enzymes through which ACR is detoxified in vivo. In this study, we have set up a panel of in vitro assays to begin to investigate whether ALDH1A1 gene therapy is effective in preventing ACR-induced Müller cell dysfunction and death.
METHODS. To mimic Müller cell dysfunction in the diabetic retina, QMMuC-1 murine Muller cells were treated with varying concentrations of ACR and cell viability, inflammatory gene expression and markers of Müller cell dysfunction and oxidative stress were evaluated using the Alamar Blue assay, qRT-PCR and Western blotting, respectively.QMMuC-1 cells were also exposed to increasing concentrations of AAV2.ALDH1A1(2800-50000 MOI) and the level of ALDH1A1 overexpression and inflammatory gene expression determined by qRT-PCR.
RESULTS. ACR triggered cell death in QMMuC-1 cells with an IC50 of 47.4 μM. Low concentrations of ACR (nM range) upregulated the mRNA expression of several inflammatory cytokines (IL-18, IL-6 and IP-10) and caused FDP-lysine accumulation on QMMuC-1 proteins. These changes were paralleled by an increase in the protein expression of the oxidative stress marker, HO-1, and a decrease in the protein expression of the K+ channel, Kir4.1, which plays an important role in Müller cell K+ homeostasis. A concentration-dependent increase in ALDH1A1 mRNA was observed by exposing the cells to varying levels of AAV2.ALDH1A1. Exposure of the cells to AAV2.ALDH1A1 did not evoke an inflammatory response in QMMuC-1 cells even at high MOIs.
CONCLUSIONS. Exposure of QMMuC-1 cells to ACR induces cellular damage similar to that observed in Müller cells in the diabetic retina. Studies are ongoing to study the feasibility of using ALDH1A1 gene therapy for preventing ACR-induced Müller cell dysfunction and death during diabetes.

Disrupted Rhythms in the Diabetic Retina: Are they Controlled by a Broken Clock?

E. Beli[1], H. Winter[1] and A. Stitt[1]

[1]*Wellcome-Wolfson Institute of Experimental Medicine, Queen's University Belfast, Belfast, UK*

DESIGN. Disruption of retina's rhythmic processes has been previously described. Particularly a phase advancement was found of many genes involved in visual perception and metabolism. We performed a metanalysis of our transcriptomic data and new electrophysiology experiments conducted in dark/dark conditions at different times during the day.
PURPOSE. Our goal is to examine whether the rhythmic disruption is driven by a disrupted clock within the retina or by a dysfunctional response to light.
METHODS. Healthy control and Ins2Akita/J diabetic mice were kept under a physiological 12h:12h light-dark

cycle until 4 months of age. Deep mRNA sequencing was conducted in retinas collected every 4 h around the day/night cycle. The rhythmic expression of clock genes (Bmal-1, clock, Npas2, Per1, Per2, Cry1, Nrd1, Rora, Rorb) and circadian controlled genes (Aanta, Nampt, cfos, fos, Drd4, Nr2e3, Nr4a1, erg1) were examined in the diabetic retina. Retina electroretinography under dark/dark conditions, which indicates circadian driven function, was performed at 4 corresponding times around the daily cycle.

RESULTS. Diabetes did not alter the phase of the circadian clock genes, nor the phase of circadian controlled genes but it reduced the amplitude of their oscillations. These data indicate that the circadian clock in the diabetic retina is entrained normally by light, but it is weakened by diabetes. Electrophysiology confirmed a weakened clock-driven oscillations that were particularly located at the inner nuclear layer, as b-wave lost rhythmicity in diabetes.

CONCLUSIONS. Although many genes are phase-shifted in the diabetic retina, the circadian clock and its output remains in phase. However the circadian clock is weakened in diabetes possible due to second order neuronal dysfunction in the retina. Other mechanisms related to light stress induction and hypoxia drive the phase shifts, creating a suboptimal alignment of the gene expression in the retina. This has been observed in other diabetic tissues and is termed internal jet lag, which over time can lead to progression of retina degeneration in diabetes.