



International  
**CXL** 2023  
Experts' Meeting

**CXL Experts' Meeting 2023  
Abstract Book**

## Table of Contents

<b>Clinical Sciences: Oral Presentations</b> .....	<b>4</b>
A Randomized Clinical Trial of Customized Corneal Cross-Linking for Keratoconus: Comparing Epi-On with Supplemental Oxygen with Epi-Off in Room Air.....	4
Adjunct Rose Bengal Photodynamic Antimicrobial Therapy for Patients with Progressive Infectious Keratitis: Outcomes of the First 100 Patients .....	5
Bowman Thickness and Imaging Profile Using Custom-Built Ultra High-Resolution OCT and its Role in Defining Progression .....	6
Can Keratoconus Disease Be Managed Medically? .....	7
Central Toxic Keratopathy After Epi-Off Corneal Cross-Linking.....	8
Customized Corneal Cross-Linking with Excimer Laser-Assisted Epithelium Removal for Progressive Keratoconus .....	9
Detection of Keratoconus with a New Corvis Biomechanical Index Optimized for the Chinese Population (cCBI) .....	10
Five-Year Follow-Up of Accelerated Versus Standard Corneal Cross-Linking in Pediatric Keratoconus.....	11
How Can Tear Biomarkers Help in Determining Cross-Linking Outcomes? .....	12
Model of Vitamin D Receptors and Its Impact on Post-Cross-Linking Haze .....	13
New Novel Technique for the Treatment of Keratoconus .....	14
Progressive High Fluence Epithelium-ON Accelerated Corneal Cross-Linking: A Novel Corneal Photo-Dynamic Therapy for Early Progressive Keratoconus .....	15
Ray-Tracing Transepithelial Excimer Laser Central Corneal Remodeling Plus Pachymetry-Guided Accelerated Corneal Cross-Linking for Keratoconus .....	16
Steroids and Cross-Linking for Ulcer Treatment (SCUT II) .....	17
Stress-Strain Index Maps: In-Vivo Assessment of Keratoconus Progression .....	18
Subclinical Keratoconus Detection and Characterization Using Motion Tracking Brillouin Microscopy .....	19
The Evaluation of Corneal Tomography Using Swept-Source Optical Coherence Tomography and Scheimpflug Imaging for Very Early Ectasia Detection.....	20
UV Light-Mediated Corneal Cross-Linking as (Lymph)-Angioregressive Pretreatment and High-Risk Corneal Transplantation .....	21
<b>Clinical Sciences: Poster Presentations</b> .....	<b>22</b>
Algorithmic Approach for Toric Implantable Collamer Lens (TICL) Planning in Post-Crosslinking Keratoconic Eyes .....	22
Antifungal Effect on <i>Candida albicans</i> and <i>Fusarium spp.</i> PACK-CXL in Combination with Low-Energy Laser Radiation (630-670 nm) in Fungal Keratitis Experiment <i>In Vivo</i> .....	23
Case series of CTK post CXL .....	24
Corneal Cross-Linking: An Alternative Treatment in Infectious Corneal Ulcers .....	25
Corneal Perforation Following Combined CXL+PRK - A Case of CTK .....	26

Epithelial Thickness Analysis Using Anterior Segment OCT in Keratoconus Patients with Very Asymmetric Ectasia .....	27
Genetic Risk Factors for Post-LASIK/PRK Ectasia .....	28
Keratometric Outcomes of Corneal Collagen Cross-Linking for Keratoconus Patients at Sussex Eye Hospital: 5-Year Results .....	29
Long-Term Keratometric and Refractive Stability After TG-PRK with CXL on Keratoconic Eyes ...	30
Optimizing UV-A Irradiation Profiles in Cross-Linking for Progressive Keratoconus: A Comparison of Outcomes After Standard Accelerated and Topography-Guided Protocols .....	31
Prevention of Vascularization Through <i>Ex Vivo</i> CXL of Corneal Donor Tissue: Evidence from Prospective Boston Kpro CXL Study.....	32
Refractive and Topographic Results of Progressive Thickness Versus Stable Thickness Intraströmical Corneal Ring Segment Implantation in Duck Type Keratoconus .....	33
Scleral Lens Fitting in a Patient After INTACTs, Cross-Linking, and Conductive Keratoplasty .....	34
The Increase in Corneal Stiffness After Accelerated Corneal Cross-Linking in Progressive Keratoconus Using Different Methods of Epithelial Debridement.....	35
Use of Corneal Cross-Linking Beyond Keratoconus: A Systemic Literature Review.....	36
Zagreb Protocol - A Combined Guided T-PRK and CXL Protocol for the Treatment of Low Visual Acuity Keratoconus .....	37
<b>Laboratory Science: Oral Presentations .....</b>	<b>38</b>
Advancing Gene Therapy for Keratoconus: Bridging the Gap Between Research and Treatment	38
Determining the impact of riboflavin replenishment on corneal cross-linking effectiveness.....	39
Enhanced transepithelial corneal cross-linking using a novel nano-biomaterial .....	40
Induction of cross-links in corneal tissue by sunlight exposure and oral riboflavin administration in rabbits: the biomechanical impact using extensometry and high-resolution OCT elastography .....	41
PACK-CXL pre-clinical research: towards translational success.....	42
Understanding the critical link of eye rubbing and inflammation by molecular pathways and its potential therapeutics .....	43
<b>Laboratory Science: Poster Presentations .....</b>	<b>44</b>
Corneal shape (Q value) changes in keratoconus (KCN) after fresh myopic intrastromal lenticular (FML) implantation .....	44
Evaluation of the effect of infiltration time on diffusion depth and rose bengal-green light corneal cross-linking efficacy .....	45
Histopathologic correlation between patients who underwent Keratoplasty after Rose Bengal Photodynamic Antimicrobial Therapy .....	46
Intraströmical Stem Cell and Telocyt Implantation for Advanced Keratoconus (KCN) .....	47
Rose Bengal Photodynamic Therapy Safety Study .....	48

## Clinical Sciences: Oral Presentations

### A Randomized Clinical Trial of Customized Corneal Cross-Linking for Keratoconus: Comparing Epi-On with Supplemental Oxygen with Epi-Off in Room Air

Sofie Elving<sup>1</sup>, Anneli Fredriksson<sup>1</sup>, Jeannette Beckman Renman<sup>1</sup>, Anders Behndig<sup>1</sup>

*1. Department of Clinical Sciences/Ophthalmology, Umeå University, Umeå, Sweden*

*Corresponding author: Prof Anders Behndig, Department of Clinical Sciences/Ophthalmology, Umeå University, Umeå, Sweden, anders.behndig@umu.se*

**Purpose:** To compare the outcomes of customized epi-on CXL with supplemental oxygen with customized epi-off CXL epi-off in room air for keratoconus.

**Setting:** Umeå University Hospital, Umeå, Sweden.

**Design:** Prospective, randomized, single-masked, intra-individually comparing study.

**Methods:** Thirty-two participants with bilateral progressive keratoconus were treated with bilateral customized CXL, 30 mW/cm<sup>2</sup>; 7.2-15 J/cm<sup>2</sup>, and were randomized to epi-on CXL with supplemental oxygen in one eye and epi-off CXL in room air in the fellow eye. Uncorrected (UDVA) and best-corrected visual acuities (BCVA), keratometry readings (Kmax and Kmean), early ocular discomfort, low-contrast visual acuities (LCVA 10% and 2.5%), manifest refractive spherical equivalent (MRSE), endothelial cell count (ECC), and adverse events were assessed through 24 months.

**Results:** Both protocols showed significant and relevant improvements at 24 months in UDVA;  $-0.16 \pm 0.24$  and  $-0.13 \pm 0.20$  logMAR ( $p < 0.001$ ), BCVA;  $-0.10 \pm 0.11$  and  $-0.10 \pm 0.12$  ( $p < 0.001$ ), Kmax;  $-1.74 \pm 1.31$  and  $-1.72 \pm 1.36$  D, ( $p < 0.001$ ), and Kmean;  $-0.72 \pm 0.59$  and  $-0.72 \pm 0.74$ , ( $p < 0.001$ ). LCVA 10% improved for both protocols ( $p < 0.001$ ), but LCVA 2.5% improved for epi-on CXL only ( $p = 0.001$ ). ECC was unaltered, and no adverse events occurred. The epi-on eyes had significantly fewer discomfort symptoms during the whole first week post-treatment ( $p < 0.05$ ).

**Conclusions:** Customized epi-on CXL with supplemental oxygen is a viable alternative to customized epi-off CXL in room air, with a larger improvement in LCVA and significantly less early ocular discomfort.

# Adjunct Rose Bengal Photodynamic Antimicrobial Therapy for Patients with Progressive Infectious Keratitis: Outcomes of the First 100 Patients

Jordan Huang<sup>1</sup>, Jaime Martinez<sup>1</sup>, Guillermo Amescua<sup>2</sup>, Darlene Miller<sup>2</sup>, Jean-Marie Parel<sup>2</sup>, Jana Tothova<sup>3</sup>

1. Department of Ophthalmology, Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, FL, USA

2. Bascom Palmer Eye Institute, University of Miami, Miami, FL, USA

3. Bascom Palmer Eye Institute, Univ of Miami Miller School of Medicine, Miami, FL, USA

Corresponding author: Dr Jordan Huang, Department of Ophthalmology, Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, FL, USA, jjh252@miami.edu

**Purpose:** To report clinical outcomes of rose bengal photodynamic antimicrobial therapy (RB-PDAT) as an adjunct treatment for severe, progressive, infectious keratitis.

**Methods:** Patients with progressive, infectious keratitis recalcitrant to standard medical treatment underwent RB-PDAT at Bascom Palmer Eye Institute from 2015 to 2023. The primary outcome was the frequency of RB-PDAT success, defined as the avoidance of therapeutic penetrating keratoplasty (TPK).

**Results:** A total of one hundred and three patients underwent RB-PDAT at Bascom Palmer Eye Institute. After exclusion criteria, 96 patients were included in this study (42 males, 54 females). Ages ranged from 15 to 87 years old. *Acanthamoeba* was the most prevalent microbe (48%; 46/96), followed by *Pseudomonas* (14%; 13/96), and *Fusarium* (11%; 11/96). The main clinical risk factor for keratitis was contact lens wear at 61% (59/96). Successful RB-PDAT (avoidance of TPK) was achieved in 76% (73/96) of cases. RB-PDAT failed in 24% (23/96) of cases. The average time to clinical resolution following RB-PDAT was  $6 \pm 10$  months. In patients that underwent TPK/OPK/DALK, corneal transplant outcomes were as follows: successful transplant (defined as a clear cornea on the last follow-up exam) was achieved in 72% (39/54) of patients, a failed transplant was found in 22% (12/54) of patients, and reinfection occurred in 6% (3/54) of patients. Amongst patients that received TPK/OPK/DALK, the average follow-up time after RB-PDAT was  $32 \pm 22$  months.

**Conclusions:** The current study results indicate that RB-PDAT shows great promise as a non-invasive adjunct treatment modality for severe, progressive infectious keratitis resistant to standard medical therapy. Patients that required a TPK maintained low reinfection rates, which may indicate further advantages to the use of RB-PDAT.

# **Bowman Thickness and Imaging Profile Using Custom-Built Ultra High-Resolution OCT and its Role in Defining Progression**

Amulya Punati<sup>1</sup>, Rohit Shetty<sup>1</sup>, Pooja Khamar<sup>1</sup>, Rahul Patil<sup>1</sup>, Abhijit Sinha Roy<sup>1</sup>

1. Narayana Nethralaya, Bangalore, India

Corresponding author: Dr Amulya Punati, Narayana Nethralaya, Bangalore, India, amulya.punati@gmail.com

**Purpose:** To investigate the Bowman's layer thickness (BLT) in eyes categorized as suspect and Keratoconus (KC) and evaluate their significance in KC progression assessment.

**Methods:** A total of 100 eyes were analyzed and categorized into two groups based on tomography and biomechanical indices. Group 1 (G1; n = 50) comprised eyes lacking clinical KC indications, while Group 2 (G2; n = 50) included eyes with either progressing or stable KC for over six months. Additionally, we included eyes with asymmetric KC, placing one eye in G2 and its fellow eye in G1. BLT profiles were mapped up to an 8 mm corneal diameter.

**Results:** Eyes in G1 displayed a uniform BLT of approximately  $15 \pm 3 \mu\text{m}$ . G2 eyes exhibited localized thinning ( $11 \pm 2 \mu\text{m}$ ), aligning with thinning observed in epithelium layer thickness (ELT) maps. The difference in BLT between the two groups was statistically significant ( $p < 0.05$ ). Some G1 eyes exhibited regional thinning (approximately  $5\text{-}7 \mu\text{m}$ ) in ELT maps but maintained a uniform BLT profile. Their corneal topography and biomechanical indices did not suggest KC, unlike G2 eyes. G2 eyes with stable KC demonstrated consistent BLT profiles during follow-up visits.

**Conclusion:** Eyes that did not progress to KC exhibited a uniform BLT profile. Our study underscores the significance of BLT profiles as a valuable parameter in corneal assessments, potentially facilitating early KC diagnosis and monitoring, thereby improving patient care and treatment outcomes. Moreover, integrating BLT data into existing artificial intelligence/machine learning models holds promise for enhancing sensitivity and specificity in classification models for KC diagnosis.

## Can Keratoconus Disease Be Managed Medically?

Ritica Mukherji<sup>1</sup>, Rohit Shetty<sup>1</sup>, Pooja Khamar<sup>1</sup>, Anisha R<sup>1</sup>

1. Narayana Nethralaya, Bangalore, India

Corresponding author: Dr Ritica Mukherji, Narayana Nethralaya, Bangalore, India, riticamukherji@gmail.com

**Purpose:** Keratoconus (KC) is a corneal ectatic disorder with a poorly understood etiology. However, the role of inflammatory biomarkers in KC pathogenesis has been well established. Activation of autophagy enables cells to protect themselves against various environmental stressors. This study aimed to assess the efficacy of trehalose, an autophagy activator, in the medical management of KC.

**Methods:** This study included 28 patients (51 eyes) with mild to moderate KC. Patients at low risk of KC progression were started on topical 3% trehalose drops four times daily. Serial corneal topography and epithelium mapping were analyzed using MS-39 (CSO), and collagen imaging was performed using polarization-sensitive optical coherence tomography (PSOCT) to assess KC status before and after treatment.

**Results:** Patients received topical trehalose for a mean duration of 8 months. Pre-treatment assessments showed a maximum keratometry (Kmax) of 50.1 D, a mean keratometry (Kmean) of 46.2 D, a median thinnest corneal thickness (TCT) of 470  $\mu\text{m}$ , stromal elevation (SE) of  $20 \pm 16$ , and a mean epithelial thickness (ET) of  $49.4 \pm 5 \mu\text{m}$ . After treatment, median Kmax, Kmean, and TCT were 48.6 D, 45.5 D, and 465  $\mu\text{m}$ , respectively, with a statistically significant change in Kmax ( $p$ -value  $< 0.05$ ). SE was reduced to  $16 \pm 16$  ( $p$ -value = 0.07), with a mean ET of  $49.3 \pm 5 \mu\text{m}$  ( $p$ -value = 1). PSOCT maps demonstrated an improvement in collagen orientation in trehalose-treated eyes.

**Conclusion:** Topical trehalose (3%) may help stabilize KC eyes, preserving topography and positively impacting collagen orientation. Trehalose and similar immunomodulators could be effective approaches for stabilizing early to moderately graded KC with low progression risk.

## Central Toxic Keratopathy After Epi-Off Corneal Cross-Linking

M. Enes Aydemir<sup>1</sup>, Emilio Torres<sup>1</sup>, Mark Hillen<sup>1</sup>, Farhad Hafezi<sup>1</sup>

1. ELZA Institute, Dietikon, Switzerland

Corresponding author: Prof Farhad Hafezi, ELZA Institute, Dietikon, Switzerland, fhafezi@elza-institute.com

**Background:** To report cases of central toxic keratopathy in patients who underwent epi-off corneal cross-linking (CXL) at a public eye clinic.

**Methods:** This case series reports on nineteen eyes from thirteen patients who developed Central Toxic Keratopathy following epi-off CXL treatment. All patients sought care at the ELZA Institute in Dietikon, Switzerland, and had previously received treatment at the Kantonsspital Luzern (eye clinic in Switzerland) between 2011 and 2016. Comprehensive ophthalmic evaluations, including slit-lamp examinations, assessment of corrected distance visual acuity (CDVA), refraction analysis, corneal topography, and optical coherence tomography (OCT) imaging, were performed on all included eyes.

**Observations:** Among the cases, OCT imaging revealed highly conspicuous scar tissue in nine eyes. One eye presented an epithelial bulla, supported by corresponding OCT documentation. Haze was present in seven eyes, while two eyes exhibited no detectable anomalies.

**Conclusions:** Despite the well-established and extensively studied nature of CXL as a treatment option for the past two decades, complications can manifest, even within the confines of a large-scale, well-regulated public eye clinic in Switzerland. Consequently, further research should be undertaken to explore potential modifications and advancements in the technique, particularly regarding less invasive approaches such as epi-on CXL.



# Customized Corneal Cross-Linking with Excimer Laser-Assisted Epithelium Removal for Progressive Keratoconus

João Quadrado Gil<sup>1</sup>, Andreia Rosa<sup>1</sup>, Maria João Quadrado<sup>1</sup>, Joaquim Murta<sup>1</sup>

1. Centro Hospitalar Universitário Coimbra, Coimbra, Portugal

Corresponding author: Dr João Quadrado Gil, Centro Hospitalar Universitário Coimbra, Coimbra, Portugal, joaomqgil@hotmail.com

**Purpose:** To evaluate the one-year visual and tomographic results of customized cross-linking using excimer laser-assisted epithelium removal and topography-guided irradiation in the treatment of progressive keratoconus.

**Setting:** Coimbra Ophthalmology Unit, Private practice, Coimbra, Portugal.

**Design:** Prospective non-randomized clinical trial.

**Methods:** Eyes with documented progressive keratoconus were enrolled. Following de-epithelization with PTK, customized UV irradiation was performed, designed as three concentric circular areas centered on the thinnest point (Mosaic System, Avedro, USA). Energy exposure was 5.4 J/cm<sup>2</sup> in the outer circle and then increased centripetally to 7.2 J/cm<sup>2</sup> and 10 J/cm<sup>2</sup>. Corrected distance visual acuity (CDVA), refractive outcomes, and Scheimpflug tomographies (Allegro Oculyzer, WaveLight, Germany) were assessed at baseline, 6 months, and 12 months postoperatively.

**Results:** This prospective study enrolled 37 eyes of 32 patients. The mean diameter for treated areas was 6.17 ± 0.80, 4.45 ± 0.47, and 2.58 ± 0.14 mm for the outer, medium, and inner circle, respectively. At the 1-year follow-up, mean CDVA improved significantly from 0.38 ± 0.19 to 0.20 ± 0.16 logMAR (p < 0.01), with 34 of the 37 eyes (91.89%) retaining or improving CDVA. Mean pre-operative minimum pachymetry decreased from 449.26 ± 41.62 μm to 443.26 ± 41.06 μm (p = 0.02). The maximal curvature (Kmax) decreased significantly from 58.50 ± 7.84 D to 57.05 ± 7.27 D (p < 0.01). After 1 year, 34 eyes (91.89%) showed no signs of progression.

**Conclusion:** One year after surgery, patients showed a significant improvement in visual acuity while achieving stabilization of disease progression.

## Detection of Keratoconus with a New Corvis Biomechanical Index Optimized for the Chinese Population (cCBI)

Riccardo Vinciguerra<sup>1</sup>, Yan Wang<sup>2</sup>, Fengju Zhang<sup>3</sup>, Xingtao Zhou<sup>4</sup>, Ji Bai<sup>5</sup>

1. Humanitas San Pio X, Milan, Italy

2. Tianjin Eye Hospital, Tianjin, China

3. Beijing Tongren Eye Center, Beijing, China

4. Eye & ENT Hospital of Fudan University, Shanghai, China

5. Bai Ji Ophthalmology, Chongqing, China

Corresponding author: Dr Riccardo Vinciguerra, Humanitas San Pio X, Milan, Italy, vinciguerra.riccardo@gmail.com

**Purpose:** The aim of this study was to introduce an optimized version of the Corvis Biomechanical Index for the Chinese population (cCBI).

**Design:** Retrospective, multicenter clinical validity-enhancement study.

**Methods:** Patients were included from seven clinics located in Beijing, Shenyang, Guangzhou, Shanghai, Wenzhou, Chongqing, and Tianjin. Logistic regression was employed to optimize the values of the constants of the CBI, based on Database 1 as the development dataset (six out of 7 clinics), to create a new version of the index named cCBI. The factors of the CBI (A1Velocity, ARTh, Stiffness Parameter-A, DARatio2mm, and Inverse Integrated Radius) and the cut-off value were kept the same (0.5). With the formation of cCBI determined, it was validated on Database 2 (1 out of 7 of the clinics).

**Results:** Two thousand four hundred and seventy-three patients (healthy and keratoconus) were included. In Database 2, the Area Under the Curve (AUC) of the cCBI was 0.985 with a 93.4% specificity and 95.5% sensitivity. In the same Dataset, the original CBI produced an AUC of 0.978 with a 68.1% specificity and 97.7% sensitivity. There was a statistically significant difference between the ROC curve of cCBI and CBI (De Long  $p=0.0009$ ).

**Conclusion:** The new cCBI for Chinese patients was shown to be statistically significantly better when compared to CBI to separate healthy from keratoconic eyes. The presence of an external validation dataset confirms this finding and suggests the use of cCBI in everyday clinical practice to aid in the diagnosis of keratoconus in the Chinese ethnicity.

# Five-Year Follow-Up of Accelerated Versus Standard Corneal Cross-Linking in Pediatric Keratoconus

Nezar Alatawna<sup>1</sup>, Boris Knyazer<sup>1</sup>

1. SOROKA Medical Center, Be'er Sheva, Israel

Corresponding author: Dr Nezar Alatawna, SOROKA Medical Center, Be'er Sheva, Israel, nezar.at99@gmail.com

**Objective:** This study aimed to assess the long-term effectiveness of accelerated (9 mW/cm<sup>2</sup>, 10 min) corneal collagen cross-linking (A-CXL) in comparison to the standard (3 mW/cm<sup>2</sup>, 30 min) approach (S-CXL) for treating Keratoconus (KC) in pediatric patients.

**Methods:** A historical cohort analysis was conducted on pediatric patients aged ≤18 years who underwent either A-CXL or S-CXL from 2010 to 2017 at two referral centers in Israel. Preoperative and postoperative assessments were conducted over a 5-year period, including a comprehensive evaluation of visual acuity, refractive errors, and keratometry data.

**Results:** Seventy-three eyes of 66 patients were analyzed (A-CXL: n = 35; S-CXL: n = 38). The A-CXL group showed a significant improvement in visual acuity compared to baseline (LogMAR UCVA: 0.82–0.35, p < 0.001; LogMAR BCVA: 0.42–0.25, p < 0.001). The improvement in visual acuity in the S-CXL group was non-significant (LogMAR UCVA: 0.8–0.67, p = 0.08; LogMAR BCVA: 0.27–0.25, p = 0.7). Kmax decreased by a mean of -0.55 ± 2.71 D following S-CXL (p = 0.7) and by -0.80 ± 1.97 D following A-CXL (p = 0.5). Thinnest pachymetry decreased following both treatments (S-CXL: by 19 ± 34 μm, p = 0.023, A-CXL: by 20 ± 39 μm, p = 0.028). Regarding Kmean, Ksteep, and Kflat, the study did not identify significant differences between either treatment group.

**Conclusion:** This study's findings demonstrate that A-CXL improves visual function when assessed over a five-year follow-up and provides valuable insights into the efficacy of A-CXL as a potential treatment option for Keratoconus in pediatric patients.

## How Can Tear Biomarkers Help in Determining Cross-Linking Outcomes?

Anisha R.<sup>1</sup>, Rohit Shetty<sup>1</sup>, Pooja Khamar<sup>1</sup>

1. Narayana Nethralaya, Bangalore, India

Corresponding author: Dr Anisha R, Narayana Nethralaya, Bangalore, India, drnisharamesh@gmail.com

**Purpose:** Keratoconus (KC) is a chronic inflammatory corneal disorder. The presence of pre-existing inflammation can lead to post-surgical sequelae, such as corneal haze, sterile infiltrates, and cross-linking (CXL) failure. This study evaluates the influence of inflammation on post-CXL outcomes and the efficacy of pre-treatment of inflammation in mitigating its impact on these outcomes.

**Methods:** This study included two arms, a retrospective and a prospective arm. The retrospective arm included 625 patients who underwent CXL. Tears were collected using Schirmer's strips, and an ELISA-based custom-built biomarker kit (Bio-M-Pathfinder) was used to analyze selected inflammatory biomarker levels. Pre-operative inflammation status was compared to post-operative outcomes. The prospective arm had 425 patients whose tears were analyzed. Patients were treated for specific biomarker-driven treatment before surgery. The incidence of CXL outcomes/complications with and without pre-treatment was compared.

**Results:** In the retrospective arm, 22 patients developed grade 2 or higher haze (incidence rate of 3.52%), 7 (1.12%) developed sterile infiltrates, 4 (0.64%) had excessive flattening, and 12 (1.92%) showed progression. Tear analysis showed that TNF- $\alpha$  and MMP-9 were 2-fold higher pre-operatively in patients with haze. IL-17 was high in sterile infiltrates, and progression cases had high MMP-9, IL-6, and IL-1 $\beta$ . Whereas in the prospective pre-treated arm (customized therapy for specific raised inflammatory biomarker), 1 (0.16%) patient developed haze, 1 (0.16%) showed progression, and no cases of sterile infiltrates or excessive flattening during the 2-year follow-up period.

**Conclusion:** Incorporating Biomarker Analysis into our keratoconus management and customizing treatment targeted therapy can help make cross-linking outcomes more predictable and with fewer complications.

## Model of Vitamin D Receptors and Its Impact on Post-Cross-Linking Haze

Anushree Bhatkal<sup>1</sup>, Sanjri Malhotra<sup>1</sup>, Pooja Khamar<sup>1</sup>, Rohit Shetty<sup>1</sup>

1. Narayana Nethralaya, Bangalore, India

Corresponding author: Dr Rohit Shetty, Narayana Nethralaya, Bangalore, India, drrohishetty@yahoo.com

**Purpose:** To investigate the influence of Vitamin D and its receptor (Vitamin D Receptor - VDR) on post-crosslinking (CXL) corneal haze in patients with keratoconus (KC). Given inflammation's established role in post-surgery haze and Vitamin D's potential to modulate inflammation via VDR, we aimed to assess VDR's functional significance in CXL-induced haze and evaluate the impact of pre-surgery Vitamin D treatment on post-CXL outcomes.

**Methods:** Corneal epithelium samples were collected from ectatic and non-ectatic zones in 30 KC patients undergoing CXL and 10 normal controls. We quantified protein and mRNA levels of VDR, lysyl oxidase (LOX), MMP9, and collagen using Western blot and qPCR. Serum Vitamin D levels were measured in CXL patients and correlated with post-CXL outcomes. Corneal haze was assessed clinically and via Pentacam-AXL Wave corneal densitometry mode. Vitamin D-deficient subjects received systemic Vitamin D supplementation.

**Results:** KC patients exhibited significantly lower VDR and LOX levels, reduced collagen levels, and elevated MMP9 levels compared to controls ( $P < 0.05$ ). VDR levels were graded lower in KC ectatic zones than in non-ectatic zones. Patients with low serum Vitamin D levels displayed reduced Vitamin D Receptors and developed grade 2 haze post-surgery, in contrast to those with normal Vitamin D levels. Conversely, Vitamin D treatment upregulated VDR and LOX expression, while decreasing MMP9 levels, thereby reducing the incidence of haze post-crosslinking.

**Conclusion:** This study highlights the crucial role of the Vitamin D-VDR axis in regulating inflammation in KC. Activating VDR through Vitamin D supplementation presents a promising strategy for enhancing post-CXL outcomes.

## New Novel Technique for the Treatment of Keratoconus

Marwan Ghabra<sup>1</sup>, John Marshall<sup>2</sup>

1. Queen Mary University of London; Consultant Ophthalmologist, Barts Health, London, UK
2. Institute of Ophthalmology, UCL & Moorfields Eye Hospital, UCLH, London, UK

Corresponding author: Mr Marwan Ghabra, Queen Mary University of London; Consultant Ophthalmologist, Barts Health, London, UK, mghabra@hotmail.com

**Purpose:** The commercially available corneal implant is a medical device designed for the treatment of moderate and advanced keratoconus. This case series describes a modified technique for using a commercially available device designed to overcome two limitations of the original technique.

**Methods:** In this study, the implantation was performed on five eyes across five patients with advanced keratoconus. These patients had previously undergone corneal collagen cross-linking more than one year before. One patient underwent corneal cross-linking two weeks before the insertion of the inlay. The lenticules are extracted from porcine tissue, which is subjected to a decellularization process, intensely cross-linked, sterilized, and packed. The implant is  $44 \pm 5$  microns in thickness and has a diameter of 7.5 to 8.0mm. A modified technique was used to insert the device into a manually constructed pocket in the posterior corneal stroma.

**Results:** The modified technique of posterior corneal stromal implantation with pocket creation via manual delamination in combination with anterior corneal collagen cross-linking is safe and results in significant improvements in the following corneal parameters: Central corneal thickness increased from  $429 \pm 22.19$  to  $463 \pm 21.89$ , the maximum keratometry decreased from  $63.4 \pm 7.73$  to  $49.92 \pm 2.37$ , and total higher-order aberrations decreased from  $3.0778 \pm 1.2$  to  $1.8924 \pm 0.379$ , decreasing Keratoconus staging from 3-4 to 1-2, and improving patient vision.

# Progressive High Fluence Epithelium-ON Accelerated Corneal Cross-Linking: A Novel Corneal Photo-Dynamic Therapy for Early Progressive Keratoconus

Cosimo Mazzotta<sup>1</sup>

1. University of Siena, Siena, Italy

Corresponding author: Prof Cosimo Mazzotta, University of Siena, Siena, Italy, cgmazzotta@libero.it

**Purpose:** To assess the preliminary clinical results of a new progressively higher fluence pulsed light Epi-ON accelerated crosslinking nomogram (PFPL M Epi-On ACXL) in the treatment of progressive keratoconus (KC).

**Setting:** Siena Crosslinking Center, Siena, Italy.

**Methods:** Prospective pilot open, non-randomized interventional study including 32 eyes of 32 young-adult patients over 26 years old with Stage I-III progressive KC undergoing PFPL M Epi-On ACXL. Riboflavin loading was performed using Paracel I 0.25% for 4 minutes and Paracel II 0.22% for 6 minutes. The New KXL I UV-A emitter (Glaukos-Avedro, Waltham, USA) was used for pulsed-light ACXL at air-room 21% oxygenation and 13 minutes of UV-A irradiation. The treatment fluence was set at 7.2 J/cm<sup>2</sup>, 8.6 J/cm<sup>2</sup>, and 10.0 J/cm<sup>2</sup> in corneas with baseline pachymetry ≤420 μm (group 1: 8 eyes), ≥ 420 μm < 460 μm (group 2, 11 eyes), and ≥ 460 μm (group 3, 13 eyes) respectively. UDVA, BCDVA, Scheimpflug corneal tomography, and AS-OCT data were collected at baseline, postoperative 1, 3, and 6 months.

**Results:** UDVA and BSCVA improved in all groups (P≤0.05). K max showed a significant decrease in the 10.0 J/cm<sup>2</sup> group (Δ - 1.68 D). The coma (HOAs) value improved significantly by the sixth month in all groups. OCT average demarcation lines were 211±19 μm in group 1, 245±23 μm in group 2, and 267±21 μm in group 3, respectively.

**Conclusions:** The preliminary results show that pachymetry-based PFPL M Epi-On ACXL nomogram stabilizes ectasia progression. Higher fluence Epi-On ACXL increases CXL penetration with better functional outcomes in the absence of complications.

# Ray-Tracing Transepithelial Excimer Laser Central Corneal Remodeling Plus Pachymetry-Guided Accelerated Corneal Cross-Linking for Keratoconus

Cosimo Mazzotta<sup>1</sup>

1. University of Siena, Siena, Italy

Corresponding author: Prof Cosimo Mazzotta, University of Siena, Siena, Italy, cgmazzotta@libero.it

**Purpose:** The aim of this study was to report the results from 12 to 96 months of a tissue-preservation algorithm based on ray-tracing-guided transepithelial excimer laser central corneal ablation (RT t-PRK) combined with individualized pachymetry-guided accelerated crosslinking (M nomogram ACXL) in young adult patients with stable keratoconus (KC).

**Methods:** Prospective interventional study including 38 eyes of 38 young adult patients (stage II KC) with a mean age of 35 years (range 26-46 years) who underwent simultaneous RT with t-PRK plus pachymetry-based ACXL in the worst eye. The treatments were performed using the iViS Suite iRES Excimer Laser (Ligi, Taranto, Italy). Ray-tracing-guided treatments were planned using the customized interactive programmed transepithelial ablation (CIPTA) 2 web software, and diagnostic data were assessed by the Precisio 2 tomographer (Ligi, Taranto, Italy) and Sirius tomographer (C.S.O., Florence, Italy). The main outcome measures included uncorrected distance visual acuity, best spectacle-corrected visual acuity, Kmax, high-order aberrations, minimum corneal thickness, and posterior elevation, with a mean follow-up of 52 months (range 12-96 m).

**Results:** The mean UDVA improved  $+3.5 \pm 1.28$  Snellen lines (SL); 38% gained  $\geq 4 \pm 1.34$  SLs, 35%  $\geq 3 \pm 1.21$  SLs, 22%  $\geq 2 \pm 1.12$  SLs, and 5%  $\geq 1 \pm 0.75$  SLs. The mean best spectacle-corrected visual acuity increased by  $+4.3 \pm 1.3$  SL. Sixty-eight percent gained  $\geq 4 \pm 0.88$  SLs and 30%  $\geq 3 \pm 0.78$  SL. No SLs were lost.

**Conclusions:** RT t-PRK plus ACXL significantly improved the quality of vision in patients with KC, preventing overcorrection and minimizing tissue consumption.



## Steroids and Cross-Linking for Ulcer Treatment (SCUT II)

N.V. Prajna<sup>1</sup>, Naveen Radhakrishnan<sup>2</sup>, Prajna Lalitha<sup>3</sup>, Guillermo Amescua<sup>4</sup>, Jennifer Rose-Nussbaumer<sup>1</sup>

1. Stanford University, Stanford, CA, USA
2. Aravind Eye Hospital, Location Unknown
3. Aravind Eye Hospital, Madurai, Tamil Nadu, India
4. Bascom Palmer Eye Institute, University of Miami, Miami, FL, USA

Corresponding author: Dr Jennifer Rose-Nussbaumer, Stanford University, Stanford, CA, USA, [rosej@stanford.edu](mailto:rosej@stanford.edu)

**Introduction:** Corneal Cross-linking (CXL) may have the potential to improve outcomes in bacterial keratitis by addressing the infection and inflammation that leads to corneal melting, scarring, and irregular astigmatism.

**Methods:** This NIH-funded international randomized, double-masked, sham, and placebo-controlled clinical trial aimed to determine the benefits of adjunctive corneal CXL and/or topical difluprednate in addition to topical antibiotic drops for the treatment of smear-positive bacterial ulcers.

**Results:** A total of 1992 patients with smear-positive bacterial ulcers were screened, and 279 participants (14%) were enrolled. Frequent reasons for exclusion from the study included corneal thickness <350  $\mu\text{m}$  (N=508; 30%), visual acuity >20/40 (N=366; 21%), follow-up (211; 12%); VA <20/200 in the unaffected eye (179; 10%), corneal perforation or scleral involvement (N=165; 10%), and prior corneal transplant or recent surgery (N=116; 7%). Of those enrolled, 96 (35%) were female. The median age was 54 (IQR 41, 63). Associated factors included manual labor (N=129; 47%), recent trauma (N=178; 65%), and contact lens wear (N=15; 5%). Median visual acuity at baseline was LogMAR 1.1 (IQR 0.54, 1.7). The geometric mean of the baseline scar size was median 2.5 mm (IQR 2.0, 3.9). Bacterial cultures grew in 182/279 (65%) and were gram-positive in 40% and gram-negative in 42% of cases.

**Conclusions:** Corneal ulceration is a significant cause of disability, particularly in developing countries, where it occurs ten times more frequently. Nearly 10% of patients presenting with unilateral infectious keratitis in our study are at risk of bilateral blindness due to pre-existing visual disability in their other eye.

## Stress-Strain Index Maps: In-Vivo Assessment of Keratoconus Progression

Vanessa Ferraro<sup>1</sup>, Ahmed Makarem<sup>2</sup>, Ahmed Elsheikh<sup>2</sup>, Paolo Vinciguerra<sup>1</sup>, Riccardo Vinciguerra<sup>3</sup>

1. Humanitas University, Milan, Italy

2. School of Engineering, University of Liverpool, Liverpool, UK

3. Humanitas San Pio X, Milan, Italy

Corresponding author: Dr Vanessa Ferraro, Humanitas University, Milan, Italy, [vanessa.ferraro@st.hunimed.eu](mailto:vanessa.ferraro@st.hunimed.eu)

**Purpose:** To assess the in-vivo biomechanical changes in progressive keratoconic patients at different time points, generating stress-strain index (SSI) maps. These SSI maps were used to investigate the biomechanical alterations associated with keratoconus progression and their potential implications for treatment planning.

**Methods:** A retrospective investigation was conducted on a dataset of progressive keratoconus patients who underwent corneal collagen cross-linking (CXL). Patients with Pentacam and Corvis ST (Oculus, Wetzlar, Germany) exams available at two different pre-CXL time points, with a minimum one-month interval between them, were included. Anonymized data were analyzed using the SSI map generator software developed by the School of Engineering, Liverpool. Statistical analysis was performed using two-sided T-tests and the Wilcoxon-Mann-Whitney test.

**Results:** The study included 30 eyes of 23 patients. The progression of keratoconus was statistically significant in terms of anterior and posterior radius of curvature, minimum corneal thickness, and Ambrosio relational thickness horizontal (ARTh) (all  $p < 0.05$ ). An alteration consistent with corneal softening of three out of four dynamic corneal response (DCR) parameters was observed, along with a reduction in the overall SSI. Notably, the SSI IN value inside the cone area showed a statistically significant decrease ( $p = 0.02$ ), as did the minimum SSI value ( $p = 0.01$ ). SSI maps illustrated variations in corneal stiffness within the cone region.

**Conclusions:** SSI worsening was found inside the area of the cone through the SSI maps, while outside the area of pathology, no statistically significant change was described. DCR parameters also showed a shift toward a softer cornea.

# Subclinical Keratoconus Detection and Characterization Using Motion Tracking Brillouin Microscopy

J. Bradley Randleman<sup>1</sup>

1. Cole Eye Institute, Cleveland Clinic, Cleveland, OH, USA

Corresponding author: Prof J. Bradley Randleman, Cole Eye Institute, Cleveland Clinic, Cleveland, OH, USA, drjbr1@gmail.com

**Purpose:** To characterize focal biomechanical alterations in subclinical keratoconus (SKC) using motion tracking (MT) Brillouin microscopy and evaluate the ability of MT Brillouin metrics to differentiate SKC eyes from normal controls.

**Methods:** Thirty eyes from 30 patients were evaluated, including 15 eyes from 15 normal patients (Controls) and 15 eyes from 15 SKC patients. All patients had clinical evaluations, Scheimpflug tomography, and MT Brillouin microscopy imaging performed using a custom-built device. Mean and Minimum (Min) MT Brillouin values within the anterior plateau region (Plateau) and anterior 150 microns (A150) were generated. Receiver operating characteristic (ROC) curves were generated for all Scheimpflug and MT Brillouin metrics evaluated to determine the area under the curve (AUC), sensitivity, and specificity for all variables.

**Results:** There were no significant differences between groups for age, sex, manifest refraction spherical equivalent refractions (MRSE), corrected distance visual acuity CDVA, K Max, or KISA% index. However, there were significant differences between Control and SKC eyes for MT Brillouin Mean Plateau (5.714 GHz vs. 5.681 GHz,  $p < 0.00000001$ ), Min Plateau (5.695 GHz vs. 5.654 GHz,  $p < 0.000000001$ ), Mean A150 (5.721 GHz vs. 5.686 GHz,  $p < 0.00000001$ ), and Min A150 (5.704 GHz vs. 5.658 GHz,  $p < 0.0000001$ ). All MT Brillouin Plateau and A150 Mean and Min metrics fully differentiated the groups (AUC = 1.0 for each), while no Scheimpflug metric fully differentiated the groups.

**Conclusions:** MT Brillouin microscopy metrics effectively characterized focal corneal biomechanical alterations in SKC eyes and clearly differentiated these eyes from controls, even when Scheimpflug metrics were not accurate in differentiation.

# The Evaluation of Corneal Tomography Using Swept-Source Optical Coherence Tomography and Scheimpflug Imaging for Very Early Ectasia Detection

Robert Herber<sup>1</sup>, Frederik Raiskup<sup>1</sup>

1. University Hospital Carl Gustav Carus, Dresden, Germany

Corresponding author: Dr Robert Herber, University Hospital Carl Gustav Carus, Dresden, Germany, robert.herber@uniklinikum-dresden.de

**Purpose:** To determine the diagnostic ability of specific corneal tomography indices to detect very early corneal ectasia using a swept-source OCT (SS-OCT, ANTERION, Heidelberg Engineering) and a Scheimpflug tomographer (RSC, Pentacam HR, Oculus).

**Methods:** Subjects in the study cohort were divided into two groups based on specific inclusion criteria. Group 1 (N=144) consisted of healthy eyes with a K-max<47 D, inferior-superior value<1.5 D, and KISA%<60. Group 2 (N=70) included eyes with normal topography (meeting the same criteria as group 1) where the contralateral eye exhibited clinical keratoconus. The main outcome measure was the area under the curve (AUC) of the ROC analysis of the Belin/Ambrosio total deviation value (BAD D) and the Pentacam Random Forest Index (PRFI) provided by RSC compared to the SCORE parameter provided by the SS-OCT. Statistical analysis was performed using the Kruskal-Wallis test and DeLong test.

**Results:** The median values of BAD D, PRFI, and SCORE were 0.99, 0.05, and -1.69 for group 1, and 1.46, 0.24, and 0.6 for group 2, respectively (all P < 0.05). When differentiating between group 2 and group 1, the ROC analysis revealed a statistically significant higher AUC for the SCORE parameter (0.903) compared to PRFI (0.763, P < 0.001) and BAD D (0.707, P < 0.001). The sensitivity and specificity were 77%/85% (SCORE) compared to 58%/90% (PRFI), and 41%/88% (BAD D).

**Discussion:** The SCORE parameter provided by SS-OCT yielded the highest AUC and, therefore, demonstrated the best diagnostic ability to detect early corneal ectasia with clinically acceptable sensitivity and specificity.

# UV Light-Mediated Corneal Cross-Linking as (Lymph)-Angioregressive Pretreatment and High-Risk Corneal Transplantation

Johanna Wiedemann<sup>1</sup>, Deniz Hos<sup>1</sup>, Claus Cursiefen<sup>1</sup>

1. University of Cologne, Department of Ophthalmology, Cologne, Germany

Corresponding author: Dr Johanna Wiedemann, University of Cologne, Department of Ophthalmology, Cologne, Germany, johanna.wiedemann@uk-koeln.de

**Background:** To improve the survival of corneal grafts in high-risk recipients, we propose a strategy of preconditioning the recipient's eye through a treatment called corneal cross-linking (CXL) before performing keratoplasty (KP). Currently, the success rate of corneal grafts is significantly lower in prevascularized eyes compared to avascular eyes. Thus, our main concept involves utilizing (lymph)angioregressive treatment (LAT) through CXL to enhance the chances of successful corneal graft survival following KP.

**Methods:** To analyze the effect of pre-transplant LAT on improving the survival of high-risk grafts, various methods (literature analysis, animal studies, small case series) were employed. The standard full-thickness penetrating procedure (KP) was used in this trial, while CXL was conducted following the accelerated protocol.

**Results:** CXL regressed CoNV before KP and resulted in a significant improvement in graft survival in mice. A small group of patients demonstrated an even more potent angioregressive effect without experiencing significant adverse effects. In the past, all studies were retrospective, lacked controls, and had small sample sizes. We have recently initiated a prospective randomized multicenter study in 8 German centers, comprising 110 patients who are affected by this condition. The study is powered to detect a reduction in the 2-year rejection rate from 50% to 25%.

**Discussion:** CXL is an effective and well-tolerated technique for reducing CoNV and shows promise as a new treatment option to enhance graft survival in high-risk KP. The initial findings of our ongoing prospective multicenter study are anticipated to be available in early 2024.

## Clinical Sciences: Poster Presentations

### Algorithmic Approach for Toric Implantable Collamer Lens (TICL) Planning in Post-Crosslinking Keratoconic Eyes

Shalu Chavan<sup>1</sup>, Rohit Shetty<sup>1</sup>, Pooja Khamar<sup>1</sup>, Amulya Punati<sup>1</sup>

1. Narayana Nethralaya, Bangalore, India

Corresponding author: Dr. Shalu Chavan, Narayana Nethralaya, Bangalore, India, chavanshalu28@gmail.com

**Purpose:** Post corneal cross-linking (CXL), various visual rehabilitation methods pose unique challenges. Toric Implantable Collamer Lenses (TICLs) show promise but exhibit variable outcomes. This study aims to develop an algorithmic approach for precise TICL implantation planning in post-CXL eyes.

**Methods:** We conducted a retrospective analysis of 50 eyes (34 post-CXL patients) undergoing TICL implantation. Seven eyes with decentered cones received intrastromal corneal rings (ICRS), 24 eyes with high higher-order aberrations underwent topography-guided PRK and simultaneous cross-linking, and 19 eyes intolerant to contact lenses received surface regularization and cross-linking. Pre-operative evaluations encompassed corneal tomography, epithelial mapping, aberrometry, and gonioscopy. Post-surgery assessments included vault measurement, safety, efficacy, and patient satisfaction.

**Results:** A two-year follow-up revealed a significant reduction in mean spherical equivalent (MRSE) from  $-7.79 \pm 4.75$  D to  $-0.52 \pm 0.90$  D, and reduced cylinder from  $-4.48 \pm 2.24$  D to  $-0.98 \pm 0.84$  D. Among the 50 eyes studied, 18 eyes demonstrated no change in visual acuity, 26 eyes exhibited a 1-line improvement, and 6 eyes experienced a 2-line gain in visual acuity. None of the eyes demonstrated a decline in visual acuity. Subsequent post-operative follow-up revealed minimal to no complaints of glare, haloes, or difficulties in daily activities for all patients.

**Conclusion:** Guided by patient selection and comprehensive evaluation, TICL implantation offers a safe and effective long-term solution for visual rehabilitation in keratoconus post-CXL. This approach enhances the quality of life by reducing reliance on spectacles or contact lenses, emphasizing the significance of tailored treatment strategies.

## **Antifungal Effect on *Candida albicans* and *Fusarium* spp. PACK-CXL in Combination with Low-Energy Laser Radiation (630-670 nm) in Fungal Keratitis Experiment *In Vivo***

Liudmyla Troichenko<sup>1</sup>, Galyna Drozhzhyna<sup>1</sup>

1. The Filatov Institute, Cornea Department, Odessa, Ukraine

Corresponding author: Dr Liudmyla Troichenko, The Filatov Institute, Cornea Department, Odessa, Ukraine, tlf2008@ukr.net

**Purpose:** Treatment of fungal keratitis is accompanied by severe consequences due to insufficiently effective local treatment and long-term systemic therapy. The search for new methods of treatment that can provide rapid and complete microbicidal and microbostatic activity is urgent.

**Methods:** The experimental study was performed on 20 eyes of 10 chinchilla rabbits weighing 3.0-4.0 kg.

**Results:** The positive antimicrobial effect of PACK-CXL in combination with low-energy laser radiation of 630-670 nm with 0.1% methylene blue (photodynamic therapy) is shown on the models of fungal keratitis of moderate degree in an *in vivo* experiment using *Candida albicans* and *Fusarium* spp. This effect is confirmed by the absence of fungal flora during microbiological examination and the achievement of epithelialization of the cornea 4.6 days earlier ( $9.2 \pm 2.77$ ) in the *Candida albicans* model and 2.4 days earlier ( $8.6 \pm 0.89$ ) in the *Fusarium* spp. model ( $p < 0.05$ ) than in the control group. In both the main and control groups, with severe keratitis, epithelialization was achieved in 50% of cases with the formation of persistent corneal clouding.

## Case series of CTK post CXL

Nancy All Raqqad,<sup>1</sup> Mohammed Alyarabi,<sup>1</sup> Mohammad Altarawneh<sup>1</sup>

1. Armed Forces Hospital, Muscat, Jordan.

Corresponding author: Dr Mohammed ALyarabi, Armed Forces Hospital, Muscat, Jordan., mohdyarabi@hotmail.com

**Purpose:** To report 5 cases of Central Toxic Keratopathy post CXL/PRK reviewed and followed up for 6 months.

**Method:** Retrospective observational case series of CTK (3 post CXL and one post PRK). 2 local cases and 2 referred cases outside KHMC seen within a 2-month period of 6 months. Details of the procedures underwent were reported. All patients were examined, treated, and followed up at 1 week, 1 month, 3 months, with refraction, UCVA, BCVA, slit lamp biomicroscopy, Pentacam, OCT anterior segment to date.

**Results:** All reported cases developed typical signs of CTK at day 3-4 post-procedure (central corneal scar, stria, and thinning associated with hypermetropic shift). No specific inciting factor was identified. They were initially treated with low-dose steroids and immunomodulators. In all cases, the corneal scar improved minimally over time, and central corneal thickness continued to progress over the first 2 months. However, visual symptoms started to disappear in all cases from 2 months onward.

**Conclusion:** No specific inciting agent was identified in all 5 cases; however, the literature documented a few possible suggestions. Possibly the accelerated mode of CXL could have an effect.



# Corneal Cross-Linking: An Alternative Treatment in Infectious Corneal Ulcers

Susanne Marx-Gross<sup>1</sup>, Faustin Ngounou<sup>2</sup>, Paul-Rolf Preussner<sup>1</sup>

1. Department of Ophthalmology, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany

2. Presbyterian Eye Service, Bafoussam, Cameroon

Corresponding author: Dr Susanne Marx-Gross, Department of Ophthalmology, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany, susanne.marx-gross@unimedizin-mainz.de

**Purpose:** For the treatment of infectious corneal ulcers in some African countries, there are no surgical options such as keratoplasty available. So cross-linking is an alternative treatment to improve vision and to avoid blindness or enucleation.

**Method:** Ten patients (age 24-73 years) with corneal ulcers were diagnosed in Bafoussam, Cameroon, Africa. The cause was usually a "dirty" corneal trauma with inadequate therapy afterward.

The patients had complaints from one month to four days before their first examination. All patients received topical antibiotics.

Visual acuity ranged from LogMAR 3 to LogMAR 0.5 with a Median of "Hand Movements" (LogMAR 2.3). All cases were treated with a modified cross-linking protocol (370 nm, 60 mW/cm<sup>2</sup>, 3 min.). Follow-up time was one month.

**Results:** In all cases, the eye could be preserved. The baseline visual acuity was in seven of the ten cases "hand movement" and worse (LogMAR 3-0.5 in all cases). In one case, full visual acuity was regained (from LogMAR 0.5 to 0). In four other cases, visual acuity was improved by at least LogMAR 0.8 and better.

In three cases, no significant increase in visual acuity was achieved, but the cornea was stabilized, and the eye was preserved.

**Conclusion:** Cross-linking represents an alternative treatment option for regions or countries where surgical options are not available to treat the infectious ulcer. The treatment protocol should be modified to achieve effective microbial elimination. In all cases, the eye could be preserved, and in seven cases, visual acuity could be improved significantly.

## **Corneal Perforation Following Combined CXL+PRK - A Case of CTK**

Doria Gabric<sup>1</sup>, Ivan Gabric<sup>1</sup>

1. Eye Clinic Svjetlost, Split, Croatia

Corresponding author: Mr Ivan Gabric, Eye Clinic Svjetlost, Split, Croatia, [ivan.gabric@svjetlost.hr](mailto:ivan.gabric@svjetlost.hr)

A patient underwent an uneventful Photorefractive Keratectomy (PRK) and same-day Corneal Cross-Linking (CXL) for Keratoconus (KC) Grade II-III in her right eye.

The initial postoperative period was uneventful, but four weeks after surgery, she exhibited Optical Coherence Tomography (OCT) signs of Corneal Thinning (CTK). However, we did not change the postoperative regimen due to poor epithelial healing.

Four months later, during a summer holiday, she experienced a spontaneous corneal perforation.

She is now scheduled for a Penetrating Keratoplasty (PKP).

# Epithelial Thickness Analysis Using Anterior Segment OCT in Keratoconus Patients with Very Asymmetric Ectasia

Tadas Naujokaitis<sup>1</sup>, Maximilian Friedrich<sup>1</sup>, Gerd U. Auffarth<sup>1</sup>, Ramin Khoramnia<sup>1</sup>, Victor A. Augustin<sup>1</sup>

1. Department of Ophthalmology, University of Heidelberg, Heidelberg, Germany

Corresponding author: Mr Tadas Naujokaitis, Department of Ophthalmology, University of Heidelberg, Heidelberg, Germany, t.naujokaitis@gmail.com

**Purpose:** To develop and test a parameter for early ectasia screening by quantifying the localized epithelial thickness differences in keratoconic eyes.

**Methods:** The dataset for parameter development included 86 eyes of 54 keratoconus patients with bilateral ectasia and 40 eyes of 20 healthy subjects. The dataset for parameter validation consisted of 42 eyes of 21 keratoconus patients with asymmetric ectasia and 21 eyes of 21 healthy subjects. Epithelial thickness maps were obtained using anterior segment optical coherence tomography, and the inter-zonal epithelial thickness differences were calculated. The developed parameter was tested in keratoconus patients with asymmetric ectasia.

**Results:** The inferior-temporal and global inter-zonal epithelial thickness differences correlated with the tomographic keratoconus progression parameters of Belin ABCD keratoconus classification ( $p < 0.001$ ) and were higher not only in eyes with tomographically significant keratoconus ( $p < 0.001$  for both parameters) but also in Scheimpflug-tomographically normal fellow eyes of keratoconus patients ( $p = 0.005$  and  $p = 0.01$ , respectively), compared to healthy controls. The median (interquartile range) inferior-temporal inter-zonal epithelial thickness differences were  $4.42 \mu\text{m}$  ( $3.13 \mu\text{m}$ ) in eyes with tomographically significant keratoconus,  $1.36 \mu\text{m}$  ( $0.85 \mu\text{m}$ ) in Scheimpflug-tomographically normal fellow eyes of keratoconus patients, and  $0.78 \mu\text{m}$  ( $0.42 \mu\text{m}$ ) in healthy controls. The median (interquartile range) global inter-zonal epithelial thickness differences were  $3.05 \mu\text{m}$  ( $1.51 \mu\text{m}$ ),  $1.31 \mu\text{m}$  ( $0.32 \mu\text{m}$ ), and  $1.07 \mu\text{m}$  ( $0.26 \mu\text{m}$ ), respectively.

**Conclusions:** The inter-zonal epithelial thickness differences can quantify the epithelial thickness changes in keratoconus. They are increased in keratoconus fellow eyes which still have a normal Scheimpflug corneal tomography and, therefore, may serve as a useful parameter to detect early ectatic changes.

## Genetic Risk Factors for Post-LASIK/PRK Ectasia

Javier Tato<sup>1</sup>, William Trattler<sup>2</sup>, Nicole Kantor<sup>3</sup>, Tori Hersman<sup>4</sup>

1. Larkin Community Hospital, South Miami, FL, USA

2. Center for Excellence In Eye Care, Kendall, FL, USA

3. Florida International University College of Medicine, Miami, FL, USA

4. Medical University of South Carolina, Charleston, SC, USA

Corresponding author: Dr Javier Tato, Larkin Community Hospital, South Miami, FL, USA, javiertatomd@gmail.com

**Background:** Technologies to identify patients at increased risk for ectasia following corneal laser refractive surgery may include the presence or absence of certain genetic variants.

**Purpose:** To identify genetic variants associated with an increased risk of ectasia after corneal refractive surgery.

**Methods:** Type of surgery (LASIK/PRK) and year performed were documented, and consent was obtained. Genetic test samples were collected. Two groups were created; a group that has not developed ectasia and a group that developed ectasia. Presence of variants in genes associated with keratoconus was recorded.

**Results:** 110 patients with a history of either LASIK or PRK were enrolled. 79 patients had developed ectasia in one or both eyes, and 39 had bilateral normal topography consistent with previous myopic laser refractive surgery. In the ectasia group, 77 of 79 patients had a history of LASIK, and 2 patients had a history of PRK. In the control group, 33 of 39 patients had a history of LASIK, and 6 had a history of PRK. Among identified genetic variants, one genetic variant, COL4A1 (Collagen type IV alpha 1), was detected in a higher percentage of ectasia patients (61 of 79 patients, 85.9%) compared to controls. Two genetic variants were found more commonly in the non-ectasia group compared to the ectasia group: COL5A1 (43.6% vs 29.6%) and PXDN (59% vs 47.9%).

**Conclusions:** Patients with ectasia following PRK or LASIK may have a different set of gene variants than patients who undergo LASIK or PRK and do not develop ectasia.

# Keratometric Outcomes of Corneal Collagen Cross-Linking for Keratoconus Patients at Sussex Eye Hospital: 5-Year Results

Mohamed Elghobaier<sup>1</sup>, Mayank Nanavaty<sup>1</sup>

1. Sussex Eye Hospital, Brighton, UK

Corresponding author: Dr Mohamed Elghobaier, Sussex Eye Hospital, Brighton, UK, mohamed.ebidalla1@nhs.net

**Purpose:** This study aims to report on the long-term keratometric results of epithelium-off corneal cross-linking (CXL) for patients with keratoconus at Sussex Eye Hospital in Brighton, United Kingdom.

**Methods:** Patients with advanced keratoconus were included in the study. Epithelial removal was performed using 70% alcohol and 2% chlorhexidine gluconate wipes (Clinell, GAMA Healthcare, UK) for 10–20 seconds. CXL was completed by applying dextran-free riboflavin (0.1%) for 10 minutes (Vibex Rapid; Avedro, Inc.), then continuous UV-A light (Avedro KXL system; Avedro, Inc.) for 30 minutes at 3 mW/cm<sup>2</sup> intensity and 5.4 J/cm<sup>2</sup> energy. Keratometric data on pre and postoperative were collected. The patients were divided into central, paracentral, and peripheral cones.

**Results:** From 2014 to 2019, 100 eyes underwent treatment with a 5-year follow-up, with central, paracentral, and peripheral cones comprising 63%, 28%, and 9%, respectively. Postoperatively, central cones showed postoperative flattening of Kmax ( $-1.54 \pm 1.94$  D) and paracentral cones ( $-0.62 \pm 1.59$  D), with no changes in peripheral cones. Patients with central cones had a preoperative posterior Kmean of  $-7.70 \pm 0.80$ , which was not significantly changed at the 1-year follow-up. In contrast, patients with paracentral and peripheral cones showed steepening. Postoperative flattening negatively correlated with paracentral and peripheral locations, while positively correlated with preoperative Kmax values in central cones. Comprehensive 5-year follow-up data will be shared upon presentation.

**Conclusion:** Postoperative flattening in central cones was consistent across all cone locations, with postoperative flattening correlated with preoperative Kmax values in central cones, but not peripheral and paracentral locations.

## Long-Term Keratometric and Refractive Stability After TG-PRK with CXL on Keratoconic Eyes

Olivier Prisant<sup>1</sup>, Elodie Pottier<sup>1</sup>

1. Clinique de la Vision, Paris, France

Corresponding author: Dr Olivier Prisant, Clinique de la Vision, Paris, France, oprisant@gmail.com

**Aim:** The aim of this retrospective study was to evaluate the visual, refractive, and keratometric stability after topoguided photoablation (TG-PRK) combined with cross-linking (CXL) performed on 154 keratoconic eyes between 2012 and 2021.

**Methods:** Inclusion criteria included stage 1 or 2 keratoconus, a pachymetry > 400  $\mu\text{m}$ , and a follow-up of at least 9 months. All eyes underwent TG-PRK associated with accelerated CXL (9 minutes, 10  $\text{mW}/\text{cm}^2$ ) with no other surgery. Mean follow-up was 24 months (minimum 9 months, maximum 9 years).

**Results:** All parameters, including BCVA, UCVA, sphere, cylinder, and maximal keratometry, were evaluated before surgery, 3 months post-operatively, and at the last visit. All parameters significantly improved between the pre-operative and the 3 months post-operative visit. There was no statistically significant difference between the 3 months and the last post-operative visit for all parameters.

**Conclusion:** In conclusion, TG-PRK combined with CXL on keratoconic eyes seems safe and efficient with a visual, refractive, and keratometric long-term stability.

# Optimizing UV-A Irradiation Profiles in Cross-Linking for Progressive Keratoconus: A Comparison of Outcomes After Standard Accelerated and Topography-Guided Protocols

João Quadrado Gil<sup>1</sup>, Telmo Cortinhal<sup>1</sup>, Andreia Rosa<sup>1</sup>, Maria João Quadrado<sup>1</sup>, Joaquim Murta<sup>2</sup>

1. Centro Hospitalar Universitário Coimbra, Coimbra, Portugal

2. jmurta@outlook.pt

Corresponding author: Dr João Quadrado Gil, Centro Hospitalar Universitário Coimbra, Coimbra, Portugal, [joaomqgil@hotmail.com](mailto:joaomqgil@hotmail.com)

**Purpose:** To compare the visual and tomographic outcomes in cross-linking treatment for progressive keratoconus, utilizing excimer-laser assisted epithelium removal and either central uniform irradiation (C-CXL) or customized, topography-guided irradiation (TG-CXL).

**Methods:** Retrospective study. Patients with progressive keratoconus who underwent TG-CXL or C-CXL. In both procedures, the epithelium was removed using phototherapeutic keratectomy (PTK) with a 50 $\mu$ m ablation within a 7.0mm optic zone. In TG-CXL, this was followed by topography-guided ultraviolet-A (UV-A) irradiation, with treatment energies ranging from 10 to 5.4J/cm<sup>2</sup>, while in C-CXL the cornea was uniformly irradiated with 6.0J/cm<sup>2</sup>. Patient data was collected at baseline, 6 and 12 months postoperatively.

**Results:** Fifty-four eyes from 48 patients were included (27 eyes for each group). Baseline characteristics were not significantly different between both groups. Kmax was significantly flattened 1 year (-0.83 $\pm$ 1.64 D; p=0.016) postoperatively for TG-CXL, but not for C-CXL (-0.46 $\pm$ 2.04 D; p=0.256). Inferior-Superior (I-S) asymmetry index decreased significantly at 1 year for TG-CXL (-9.70 $\pm$ 12.30 D; p<0.001), but not for C-CXL (-3.69 $\pm$ 11.69 D; p=0.113). The BCVA improved significantly at 1 year (difference to baseline: TG-CXL -0.13 $\pm$ 0.14 logMAR; p<0.001 and C-CXL -0.24 $\pm$ 0.38 logMAR; p=0.018. No difference between groups; p=0.445), while there was a significant myopic increase in spherical refractive error in both groups (difference to baseline: TG-CXL -1.05 $\pm$ 2.08 D; p=0.017 and C-CXL -0.90 $\pm$ 1.49 D; p=0.025).

**Conclusion:** One year after surgery, TG-CXL leads to greater Kmax flattening and topographic regularization than C-CXL. Both procedures lead to improved visual acuity and a myopic shift, with no superiority among the two.

## **Prevention of Vascularization Through *Ex Vivo* CXL of Corneal Donor Tissue: Evidence from Prospective Boston Kpro CXL Study**

Joseph Ciolino<sup>1</sup>, Ana Roldan<sup>1</sup>, Rohan Singh<sup>1</sup>, Nikolay Boychev<sup>1</sup>, Sofia De Arrigunaga<sup>2</sup>

1. Massachusetts Eye and Ear, Boston, MA, USA

2. sxd1460@miami.edu

Corresponding author: Dr Joseph Ciolino, Massachusetts Eye and Ear, Boston, MA, USA,  
joseph\_ciolino@meei.harvard.edu

**Purpose:** The purpose of this study was to investigate the impact of *ex vivo* corneal cross-linking (CXL) on corneal neovascularization (CNV) in eyes receiving cadaveric corneal donor carrier tissue for the Boston Keratoprosthesis (BKPro).

**Methods:** We conducted a masked analysis of eyes randomized into two groups: one group received cadaveric corneal donor carrier tissue for BKPro and underwent *ex vivo* corneal cross-linking (CXL), while the other group received the same tissue without CXL. Photographic quantification was employed to compare CNV metrics between the two groups.

**Results:** Our study revealed that CNV levels were significantly lower in eyes that received donor corneas subjected to CXL compared to those without CXL. This statistical difference suggests that the application of CXL to donor corneas may confer resistance to corneal neovascularization.

**Conclusion:** In conclusion, our findings indicate that *ex vivo* corneal cross-linking (CXL) of donor corneas for the Boston Keratoprosthesis (BKPro) is associated with a reduced risk of corneal neovascularization (CNV).



# Refractive and Topographic Results of Progressive Thickness Versus Stable Thickness Intraströmial Corneal Ring Segment Implantation in Duck Type Keratoconus

Efehan Coşkunseven<sup>1</sup>

1. Dünya Eye Hospital, Istanbul, Turkey

Corresponding author: Mr Efehan Coskunseven, Dünya Eye Hospital, Istanbul, Turkey, efekanc@gmail.com

**Methods:** In this retrospective case series, patients who had unsatisfactory spectacle-corrected vision and contact lens-intolerance because of advanced duck-type keratoconus underwent ICRS implantation. 32 eyes with progressive thickness ICRS implantation and 26 eyes with stable thickness ICRS implantation were included in the study. The complete refractive assessment, including the measurement of uncorrected visual acuity, best-corrected visual acuity, spherical equivalent, was performed preoperatively and postoperatively. The difference between maximum and minimum keratometry (K) in a 3 mm central area was evaluated.

**Results:** The mean age was 26.5 years in the progressive ICRS group and 28.4 years in the stable ICRS group. The mean keratometry (K) decreased to 45.8 D postoperatively from 48.1 D preoperatively in the progressive thickness ICRS group. The mean K decreased to 46.8 D postoperatively from 49.8 D preoperatively in the stable thickness ICRS group. The reduction of the mean K in the progressive thickness ICRS group was comparable in both groups. However, the difference between maximum and minimum keratometry in a 3 mm central area was 3.52 D preoperatively in the progressive ICRS group and decreased to 1.82 D postoperatively, while it decreased from 3.07 D preoperatively to 1.80 D postoperatively in the stable thickness group.

**Conclusion:** The implantation of the progressive thickness ICRS is more effective in correcting the central irregularity of duck-type keratoconus than the implantation of the stable thickness ICRS.

# Scleral Lens Fitting in a Patient After INTACTs, Cross-Linking, and Conductive Keratoplasty

Stephanie Frankel<sup>1</sup>

1. Bascom Palmer Eye Institute, Miami, FL, USA

Corresponding author: Dr Stephanie Frankel, Bascom Palmer Eye Institute, Miami, FL, USA, s.frankel1@miami.edu

**Purpose:** To report a case of a 38-year-old male with a presenting history of keratoconus in both eyes, with progression noted through topographical scan over a 2-year period. The patient went on to receive Holcomb C3-R corneal cross-linking with INTACTs in both eyes, as well as Conductive Keratoplasty (CK) in his left eye. He later developed implant extrusion in his left eye four years later that resulted in implant removal. He was unsuccessfully fit with scleral lenses by an outside provider, secondary to poor vision and mid-day fogging. The patient was refitted at our facility with a 17.5 mm diameter scleral lens, designed using corneo-scleral topography and image-guided lens design, resulting in a visual acuity of 20/20 and 20/25. Shortly after lens dispensing, the patient developed severe allergic conjunctivitis and dermatitis from the scleral lens material. After changing the lens material to Hfocon A, the patient had a complete resolution of his allergic reaction resulting in successful all-day scleral lens wear.

**Conclusions:** Image-guided scleral lenses could be tried in patients who have received corneal cross-linking and ICRS and have failed previous scleral lens fittings or are intolerant to corneal RGP or soft contact lenses. This should be done before subjecting such patients to keratoplasty.

# The Increase in Corneal Stiffness After Accelerated Corneal Cross-Linking in Progressive Keratoconus Using Different Methods of Epithelial Debridement

Robert Herber<sup>1</sup>, Frederik Raiskup<sup>1</sup>

1. University Hospital Carl Gustav Carus, Dresden, Germany

Corresponding author: Dr Robert Herber, University Hospital Carl Gustav Carus, Dresden, Germany, robert.herber@uniklinikum-dresden.de

**Purpose:** To investigate the corneal stiffening effect after epithelium-off accelerated corneal cross-linking (A-CXL (9\*10)) with mechanical epithelial debridement (group 1), transepithelial phototherapeutic keratectomy (group 2), and wavefront-guided photorefractive keratectomy (group 3), correcting high order aberrations (HOA), in progressive keratoconus (KC).

**Methods:** This was a retrospective, interventional, and non-randomized study. KC Patients in whom CXL was indicated were treated with different types of epithelial removal. Epithelium was removed using a hockey knife in group 1. For group 2 (n=39) and group 3 (n=22), epithelium thickness was measured and ablated using an excimer laser, whereas a correction of HOA were performed only in group 3. Corneal biomechanics (integrated invers radius (IIR) and stress-strain index (SSI)) and corneal tomography (thinnest corneal thickness (TCT)) were assessed with Corvis ST and Pentacam HR before and one month after-CXL. For the analysis of the group comparisons, an ANOVA and a paired t-test were used.

**Results:** Corneal tomography did not differ between the groups preoperatively ( $P>0.05$ ). TCT decreased post-CXL statistically significantly by -12.38, -20.67, and -42.82 $\mu$ m for group 1, 2, and 3, respectively (all  $P<0.05$ ). Nonetheless, corneal biomechanical stiffening was found in all three groups indicated by an increased SSI and a decreased IIR (all  $P<0.05$ ). For group 3, the HOA improved statistically significantly ( $P<0.001$ ). Between the groups, there were no significant differences in biomechanical parameters but TCT was significantly reduced after laser ablation.

**Discussion:** Corneal stiffening occurs after A-CXL independently of the epithelium removal procedure. The reduction of HOA indicates a potential improvement in visual acuity.

# Use of Corneal Cross-Linking Beyond Keratoconus: A Systemic Literature Review

Asaf Achiron<sup>1</sup>

1. Tel Aviv Medical Center, Tel Aviv, Israel

Corresponding author: Dr Asaf Achiron, Tel Aviv Medical Center, Tel Aviv, Israel, achironasaf@gmail.com

**Purpose:** The success of corneal collagen cross-linking in altering keratoconus' clinical course has driven a search for further uses of this procedure. This literature review aims to analyze the scientific evidence available for cross-linking in the management of ophthalmic diseases other than progressive keratoconus or ectasia induced by corneal refractive procedures.

**Methods:** A systemic literature review.

**Results:** We reviewed 97 studies. We found that collagen cross-linking can limit the progression of several other corneal ectasias, thus reducing and limiting the need for keratoplasty. Collagen cross-linking can be considered for a moderate degree of bacterial keratitis or when the organism is unidentified, which is refractive to antibiotics alone.

**Conclusion:** Corneal collagen cross-linking can be used effectively to promote care not only for keratoconus.

## Zagreb Protocol - A Combined Guided T-PRK and CXL Protocol for the Treatment of Low Visual Acuity Keratoconus

Doria Gabric<sup>1</sup>, Alma Bišćević<sup>1</sup>, Mateja Jagić<sup>1</sup>, Ivan Gabric<sup>1</sup>, Lucija Žerjav<sup>1</sup>

1. Eye Clinic Svjetlost, Split, Croatia

Corresponding author: Mr Ivan Gabric, Eye Clinic Svjetlost, Split, Croatia, ivan.gabric@svjetlost.hr

**Purpose:** To present a case series of 48 eyes that underwent a combined corneal collagen cross-linking (CXL) and wavefront-guided photorefractive keratectomy (PRK) protocol for the treatment of keratoconus, with a focus on reducing anterior elevation maps to improve visual outcomes.

**Methods:** The study included 48 eyes of patients with keratoconus ranging from grade 1 to 4. Inclusion criteria were reduced corrected distance visual acuity (CDVA) and patient-reported visual issues with glasses or soft contact lenses. Various diagnostic tools were used for patient screening. Custom laser ablation was planned considering epithelial variability, and all procedures were performed on the same day, with CXL followed by custom laser ablation. Ablations were limited to 15% of corneal tissues, excluding the epithelium. The Hafezi sub-400 protocol was used for corneas with a thickness below 400 microns.

**Results:** Before the procedure, the average uncorrected visual acuity (UCVA) was 0.77 logMAR, while the average best-corrected visual acuity (BCVA) was 0.22 logMAR. After the procedure, the average BCVA improved to 0.09 logMAR. The average corneal higher-order aberrations (HoA) decreased from 2.07 root mean square (RMS) before the procedure to 1.35 RMS after. Coma also decreased significantly, with an average of 1.6 RMS before the procedure and 0.89 RMS after. The mean follow-up period was 1 year.

## Laboratory Science: Oral Presentations

### Advancing Gene Therapy for Keratoconus: Bridging the Gap Between Research and Treatment

Pooja Khamar<sup>1</sup>, Rohit Shetty<sup>1</sup>, Arkasubhra Ghosh<sup>1</sup>

1. Narayana Nethralaya, Bangalore, India

Corresponding author: Dr Pooja Khamar, Narayana Nethralaya, Bangalore, India, dr.poojakhamar@gmail.com

**Purpose:** Keratoconus is characterized by progressive stromal weakness and thinning, with severity inversely related to lysyl oxidase (LOX) enzyme levels. This study investigates the potential benefits of enhancing LOX expression in keratoconic tissues and aims to establish the safety and efficacy of recombinant adeno-associated virus (AAV)-mediated LOX gene delivery as a therapeutic approach for keratoconus.

**Methods:** Human corneal lenticules from SMILE surgeries were used after ethics approval. AAV vectors expressing LOX were generated and purified. Human lenticules and mouse corneas were transduced with AAV.LOX, while AAV.GFP served as controls. LOX, Collagen I, Collagen IV, and MMP9 levels were assessed in human tissues (4 weeks) and mouse corneas (6 weeks) using immunofluorescence and mRNA analysis. Atomic force microscopy (AFM) measured corneal strength post AAV injection.

**Results:** AAV.LOX transduction in mice was safe without ocular/systemic complications. AAV.LOX efficiently transduced corneal fibroblasts in humans and mice. Elevated LOX correlated with increased collagen and ECM-related genes, e.g., Fibronectin and  $\alpha$ -SMA, and reduced MMP9 levels. Higher LOX expression was associated with lower baseline levels of MMP9, indicating a reduction in inflammation. AFM revealed an increased elastic modulus post AAV.LOX.

**Conclusion:** Recombinant AAV-mediated LOX therapy proves to be a safe and effective approach for treating ectatic corneas. Augmenting LOX enhances collagen expression and reduces MMP9 levels, halting the thinning process and strengthening the cornea. The reduction in MMP9 levels can alleviate the inflammatory environment, offering a promising avenue for keratoconus treatment.

## Determining the impact of riboflavin replenishment on corneal cross-linking effectiveness

Siân R. Morgan<sup>1</sup>, David P.S. O'Brart<sup>2</sup>, Andrew J. Quantock<sup>1</sup>, Keith M. Meek<sup>1</sup>, Sally Hayes<sup>1</sup>

1. Structural Biophysics Research Group, School of Optometry and Vision Sciences, Cardiff University, Cardiff, UK

2. Keratoconus Research Institute, Department of Ophthalmology, St Thomas' Hospital, London, UK

Corresponding author: Dr Siân R. Morgan, Structural Biophysics Research Group, School of Optometry and Vision Sciences, Cardiff University, Cardiff, UK, morgans51@cardiff.ac.uk

**Purpose:** To assess the impact of riboflavin replenishment during corneal cross-linking (CXL).

**Methods:** 51 porcine eyes were assigned to 6 groups. Group 1 remained untreated. Groups 2-6 received a 16-minute application of 0.1% riboflavin/HPMC. Group 3 was then exposed to 9 mW/cm<sup>2</sup> UVA for 10 minutes. Groups 4-6 were also exposed to 9 mW/cm<sup>2</sup> UVA for 10 minutes but received a midway replenishment of riboflavin during continuous irradiation (Group 4) or in association with a 1-minute (Group 5) or 2-minute (Group 6) break in UVA exposure. Central corneal thickness (CCT) was recorded before and after treatment. Trepined 8.0 mm central corneal buttons were subjected to 0.3% collagenase digestion at 37°C and monitored at 1-hour intervals to determine the time required for complete digestion.

**Results:** A midway replenishment of riboflavin during continuous UVA exposure maintained CCT during treatment, whereas no riboflavin replenishment or riboflavin replenishment accompanied by a 1–2-minute break in UVA exposure resulted in a small but significant reduction in CCT ( $P < 0.01$ ). All CXL-treated corneas displayed a 2-5-fold increase in resistance to collagenase digestion compared to non-irradiated corneas. However, no significant differences in enzymatic resistance were detected between any of the CXL groups.

**Conclusions:** All CXL protocols were effective at enhancing the resistance of the cornea to collagenase digestion. The inclusion of a midway replenishment of riboflavin did not significantly increase CXL effectiveness but, with continuous UVA exposure, did help to maintain tissue thickness during treatment.

## Enhanced transepithelial corneal cross-linking using a novel nano-biomaterial

Jinhai Huang<sup>1</sup>, Mei Yang<sup>2</sup>, Colm McAlinden<sup>1</sup>, Keith M. Meek<sup>3</sup>, Xingtao Zhou<sup>4</sup>

1. Corneo Plastic Unit & Eye Bank, Queen Victoria Hospital, East Grinstead, UK

2. Fudan University, Shanghai, China

3. Structural Biophysics Research Group, School of Optometry and Vision Sciences, Cardiff University, Cardiff, UK

4. Eye Institute and Department of Ophthalmology, Eye & ENT Hospital, Fudan University, Shanghai, China

Corresponding author: Dr Mei Yang, Eye Institute and Department of Ophthalmology, Eye & ENT Hospital, Fudan University, Shanghai, China, 997682582@qq.com

**Purpose:** Hibiscus-like RF@ZIF-8NF composites were constructed with the purpose of achieving a good TE-CXL effect while preserving the epithelium, providing a new strategy for the treatment of keratoconus.

**Materials and Methods:** Using 5-phosphoric acid-riboflavin (RF), 2-methylmimi, and zinc nitrate as raw materials, RF@ZIF-8 composites were obtained by adjusting reaction conditions. Detailed material characterization, in vitro, and in vivo biological evaluations of the obtained RF@ZIF-8 composites were carried out.

**Results:** The doping of RF in an appropriate range has little effect on the phase structure of ZIF-8, while the morphology and size of the composites can be adjusted by changing reaction factors. Under the same conditions, various experimental results indicated that the obtained RF@ZIF-8 composites have excellent corneal permeability, and the RF content in the stroma was higher than that in other groups. The biocompatibility evaluation showed that the RF@ZIF-8 NF nanocomposites had excellent biocompatibility and no obvious damage to the cornea. The in vivo TE-CXL effect of the RF@ZIF-8 composite was comparable to, or even slightly better than that of SCXL.

**Conclusion:** The constructed hibiscus-like RF@ZIF-8NF composites have an excellent TE-CXL effect with the preservation of the epithelium. Their TE-CXL effect is comparable to that of SCXL, or even slightly better than that. It is expected to become a new type of TE-CXL nano-drug, providing a new strategy for the treatment of keratoconus. This research has high scientific research value and opens up a new direction for the development of CXL.



# Induction of cross-links in corneal tissue by sunlight exposure and oral riboflavin administration in rabbits: the biomechanical impact using extensometry and high-resolution OCT elastography

Emilio Torres-Netto<sup>1</sup>, Sabine Kling<sup>2</sup>, M. Enes Aydemir<sup>1</sup>, Nanji Lu<sup>1</sup>, Farhad Hafezi<sup>1</sup>

1. ELZA Institute, Dietikon, Switzerland

2. Federal Institute of Technology (ETH) Zurich, Zurich, Switzerland

Corresponding author: Dr Emilio Torres-Netto, ELZA Institute, Dietikon, Switzerland, emilio.torres@cabmm.uzh.ch

**Purpose:** The range of sunlight extends from the UV-C spectrum to the infrared, including UV-A at 365 nm. Previous experiments have shown that ex vivo exposure of isolated porcine corneas soaked in riboflavin leads to a stiffening effect similar to the one observed in classic corneal cross-linking. Here, we assessed whether long-term administration of oral riboflavin, combined with extended exposure to natural sunlight, may lead to a stiffening effect in the corneas of free-moving rabbits. Biomechanical changes were analyzed using OCT elastography and stress-strain extensometry.

**Methods:** Sixteen Male New Zealand White rabbits were used. First, four rabbits received riboflavin orally, and stromal riboflavin concentration was estimated. Twelve additional rabbits were divided into two groups: the riboflavin group received vitamin B2 and sunlight exposure, while the control group was exposed to sunlight only. OCT elastography was conducted by applying a 5 mmHg change in ambient pressure. The cornea was analyzed using stress-strain extensometry.

**Results:** After relaxation, control and riboflavin conditions had a stress of  $152 \pm 11.5$  kPa and  $146 \pm 7.0$  kPa ( $p=0.57$ ). The mean elastic modulus between 0.1 and 0.2 strain was 4.1 and 4.0 MPa ( $p=0.870$ ). In elastography, the posterior half of the riboflavin cornea presented a higher strain amplitude compared to the control cornea (8.1‰ versus 3.8‰,  $p=0.03$ ), suggesting a lower stiffness in the former.

**Conclusions:** Oral riboflavin and reduced sunlight exposure in vivo did not significantly improve the corneal stiffness of rabbit corneas. Interestingly, animals that received riboflavin and were exposed to sunlight showed a trend toward softening in the posterior stroma.

## PACK-CXL pre-clinical research: towards translational success

Malwina Kowalska<sup>1</sup>, Simon Pot<sup>2</sup>, Sonja Hartnack<sup>1</sup>

1. Veterinary Epidemiology, University of Zurich (UZH), Zurich, Switzerland

2. Veterinary Ophthalmology, University of Zurich (UZH), Zurich, Switzerland

Corresponding author: Dr Malwina Kowalska, Veterinary Epidemiology, University of Zurich (UZH), Zurich, Switzerland, malwina.kowalska@uzh.ch

**Background:** PACK-CXL is a relatively new pre-clinical research domain, with diverse in vitro conditions, animal models, and PACK-CXL protocols tested. A systematic mapping of pre-clinical PACK-CXL studies seems lacking but is essential to guide future research efforts.

Scoping and systematic reviews are critical for summarizing evidence and facilitating translation [1], evaluating how the choice of model, randomization, and masking procedures impact observed treatment effect sizes [2, 3].

**Methods:** A scoping review protocol was written according to PRISMA guidelines [4]. Embase, MEDLINE, Scopus, and Web of Science databases were searched to identify eligible records. Extracted information included:<sup>1</sup> PACK-CXL protocol characteristics;<sup>2</sup> infectious pathogens tested;<sup>3</sup> study design and experimental settings; and<sup>4</sup> endpoints used to determine antimicrobial and tissue stabilizing effects of PACK-CXL treatment.

**Results:** 176 records met the inclusion criteria (of 1656 unique records identified in the search). Various PACK-CXL protocols were investigated, using 92 heterogeneous endpoints. Bacterial solutions and infectious keratitis rabbit models were most frequently used to assess the antimicrobial effects of PACK-CXL. A large variety of models was used to assess the tissue stabilizing effect.

Shortcomings were observed in experimental design reporting, including intervention (PACK-CXL protocol) details, pain control strategies, and clinical scoring systems in live animal studies.

**Conclusions:** Incomplete reporting and a large heterogeneity of endpoints precluded knowledge synthesis, for example, through a systematic review. If pre-clinical PACK-CXL studies are to guide future translational research, cross-disciplinary efforts are needed to establish and promote core sets of endpoints relevant to PACK-CXL.

## Understanding the critical link of eye rubbing and inflammation by molecular pathways and its potential therapeutics

Sanjri Malhotra<sup>1</sup>, Rohit Shetty<sup>1</sup>, Pooja Khamar<sup>1</sup>, Nithin Kiran<sup>1</sup>, Nimisha Rajiv Kumar<sup>1</sup>

1. Narayana Nethralaya, Bangalore, India

Corresponding author: Dr Rohit Shetty, Narayana Nethralaya, Bangalore, India, drrohitshetty@yahoo.com

**Purpose:** It is known that keratoconic eyes have inflammation with many reporting increased eye rubbing. Therefore, we investigated the role of a topical eye formulation on dysregulated corneal extracellular matrix factors in mice corneas under eye rubbing and inflammatory stress.

**Methods:** C57BL/6 mice were grouped into controls and LPS (1ug/mL, 3 days pre-treatment) + eye rub (ER, 12 rubs each time/day using a sterile cotton swab) induced inflammation group. From Day 4 to Day 10, inflammation group mice were subjected to closed-eye rub + LPS followed by topical treatment with Trehalose (3% w/v), Batimastat (50uM), or their combination. Gene expression and protein levels of LOX, MMP9, and collagens were measured in the enucleated corneas.

**Results:** Mice corneas stimulated with LPS + ER showed intact corneal layers with a significant reduction in LOX and collagen levels compared to controls. Trehalose, Batimastat, or their combination treatments restored LOX expression. Concurrently, the treatment reduced MMP9 and increased collagen expression. Furthermore, the treatments did not show any toxicity or morphological changes to the corneal layers.

**Conclusion:** We have established a new model for keratoconus molecular pathology. Trehalose and Batimastat treatments restore LOX expression in this in vivo model of inflammatory eye rubbing, providing a potential new therapy for keratoconus.

## Laboratory Science: Poster Presentations

### Corneal shape (Q value) changes in keratoconus (KCN) after fresh myopic intrastromal lenticular (FML) implantation

Faruk Semiz<sup>1</sup>, Ceren Ece Semiz<sup>1</sup>, Njomza Hima Musa<sup>1</sup>, Zekeriya Alp Demirsoy<sup>1</sup>

1. Eye Hospital, Pristina, Kosovo

Corresponding author: Dr Faruk Semiz, Eye Hospital, Pristina, Kosovo, faruksemiz@yahoo.com

**Purpose:** To demonstrate that the corneal Q value changes from conical to ellipsoid after intrastromal FML implantation in corneal cross-linked patients with keratoconus.

**Methods:** Thirty eyes of 15 patients diagnosed with keratoconus who underwent bilateral corneal cross-linking (CXL), aged 18 to 28 years, and whose central corneal thickness varied between 420 and 450  $\mu\text{m}$  were included in this study. FML implants were placed in all eyes using the SMILE surgery. Changes in corneal Q value at 1, 3, 6 months, and 1 year after implantation were examined.

**Results:** Preoperative corrected distance visual acuity (CDVA) improved from  $0.86 \pm 0.22$  logMAR (range: 0.40 to 1.10 logMAR) to  $0.47 \pm 0.09$  logMAR (range: 0.30 to 0.52 logMAR) ( $P < .001$ ) at the 1-year postoperative mark. Preoperative central corneal thickness (CCT) increased from 420 to 530  $\mu\text{m}$  ( $P < .001$ ). The preoperative Q value increased from -0.80 to -0.48 ( $P < .001$ ).

**Conclusion:** The corneal Q value changes from conical to ellipsoid after FML implantation in corneal cross-linked patients with keratoconus. This technique is safe, economical, and reliable, increasing visual acuity, corneal thickness, and promoting the renewal of healthy keratocytes and telocyte-like cells, crucial for stromal regeneration.

**ClinicalTrials.gov identifier:** NCT04591587.

## Evaluation of the effect of infiltration time on diffusion depth and rose bengal-green light corneal cross-linking efficacy

Jinhai Huang<sup>1</sup>, Rongrong Gao<sup>2</sup>, Keith M. Meek<sup>1</sup>

1. Structural Biophysics Research Group, School of Optometry and Vision Sciences, Cardiff University, Cardiff, UK

2. National Clinical Research Center for Ocular Diseases, Eye Hospital, Wenzhou Medical University, Wenzhou, China

Corresponding author: Dr Jinhai Huang, Eye Institute and Department of Ophthalmology, Eye & ENT Hospital, Fudan University, Shanghai, China, vip999vip@163.com

**Purpose:** To explore the diffusion depth and green light corneal cross-linking efficacy of different rose bengal (Rb) infiltration times in rabbit eyes.

**Methods:** Twenty-eight fresh rabbit eyes were deepithelialized and infiltrated in 0.1% Rb solution for 2 to 30 minutes. Corneal frozen sections were cut, and Rb diffusion depth was observed under the confocal microscope. Furthermore, 36 rabbits were randomly divided into 8 groups according to the type of treatment (control, Rb infiltration only without irradiation, rose bengal/green light (RGX) for different infiltration times, or riboflavin/UV radiation (UVX)). The corneas' resistance to keratolysis and biomechanical properties were measured after treatment.

**Results:** After 2, 10, 20, and 30 minutes of infiltration, Rb penetration depths in the corneal stroma were about 100, 150, 200, and 270  $\mu\text{m}$ , respectively. The time for complete digestion of the RGX 10 minutes ( $14.0 \pm 1.4$  hours), RGX 20 minutes ( $18.8 \pm 1.1$  hours), and UVX ( $51.2 \pm 7.2$  hours) groups was statistically greater than the control ( $7.2 \pm 1.1$  hours). At 10% extension, the Young's modulus of the RGX 20 minutes ( $36.59 \pm 4.90$  MPa) and UVX ( $40.89 \pm 2.57$  MPa) groups was statistically greater than the control ( $21.76 \pm 5.69$  MPa).

**Conclusion:** Appropriately prolonging the infiltration time can increase the Rb diffusion depth and the Rb content in the corneal stroma. Furthermore, RGX for 20 minutes showed the best cross-linking efficacy, albeit not as good as UVX

## **Histopathologic correlation between patients who underwent Keratoplasty after Rose Bengal Photodynamic Antimicrobial Therapy**

Juan Carlos Navia<sup>1</sup>, Jaime Martinez Martinez<sup>1</sup>, Jordan Huang<sup>1</sup>, Guillermo Amescua<sup>1</sup>, Jean-Marie Parel<sup>1</sup>

1. Bascom Palmer Eye Institute, Miami, Florida, USA

Corresponding author: Dr Jaime Martinez Martinez, Bascom Palmer Eye Institute, Miami, Florida, USA, [jaimedavidmartinez@gmail.com](mailto:jaimedavidmartinez@gmail.com)

**Purpose:** To compare the histopathologic features of Acanthamoeba isolates recovered from patients who underwent Therapeutic Penetrating Keratoplasties (TPK), Optical Penetrating Keratoplasties (OPK), and Deep Anterior Lamellar Keratoplasties (DALK) after adjunct treatment with Rose Bengal Photodynamic Antimicrobial Therapy (RB-PDAT).

**Methods:** A retrospective review of patients diagnosed with Acanthamoeba keratitis at the Bascom Palmer Eye Institute between August 2015 and August 2022 who underwent TPKs and OPKs after RB-PDAT. Host corneas were analyzed after keratoplasty for Acanthamoeba cyst depth, number of cysts, and average corneal thickness.

**Results:** Seventeen eyes of 17 patients were included (43.7±19.9 years old; 70.5% female). Of those, 8 patients (47.0%) required TPKs, and 9 (52.9%) underwent OPKs/DALKs. Of the 8 patients that underwent TPK, 6 patients were positive for cysts on cornea tissue pathology analysis. Amongst these patients, the average Acanthamoeba cyst depth from Descemet's layer was 42.0±52.5 mm. The average number of grading cysts was 1.3±0.7. The average corneal button thickness was 661.7±106.5 mm. The rate of reinfection after TPK was 12.5% (1/8 patients). Of the 9 patients that underwent OPK/DALK, 3 patients were positive for cysts on corneal tissue pathology analysis. Amongst the 3 patients, the average Acanthamoeba cyst depth from Descemet's layer was 261.7±222.7 mm. The average number of grading cysts was 1.3±0.9. The average corneal button thickness was 474.2±126.6 mm.

**Conclusions:** Pathological analysis of host corneal tissue harvested from patients who underwent RB-PDT for Acanthamoeba infections revealed C:Deeper cyst depth in the cornea in patients undergoing TPK compared to OPK/DALK.

## **Intrastromal Stem Cell and Telocyt Implantation for Advanced Keratoconus (KCN)**

Faruk Semiz<sup>1</sup>, Ceren Ece Semiz<sup>1</sup>, Njomza Hima Musa<sup>1</sup>, Zekeriya Alp Demirsoy<sup>1</sup>, Gamze Tanriverdi<sup>1</sup>

1. Eye Hospital, Pristina, Kosovo

Corresponding author: Dr Faruk Semiz, Eye Hospital, Pristina, Kosovo, faruksemiz@yahoo.com

**Purpose:** To investigate the development of new intrastromal histological structures using transmission electron microscopy, immunofluorescence, and light microscopy after fresh myopic human lenticular (FML) implantation with stem cells and telocytes in advanced Keratoconus (KCN).

**Methods:** Sixty eyes with advanced KCN indicated for corneal transplantation were included in this study. FML implants were placed in all eyes through small-incision lenticule extraction (SMILE) surgery. Lenticular implants were extracted from patients with myopic refractive errors of the cornea, untreated KCN, and treated KCN following 1, 2, and 3 years of surgery. These five lenticular samples were examined under the electron microscope, immunofluorescence, and light microscopy and compared.

**Results:** Healthy keratocytes and telocyte-like cells were observed in samples obtained 3 years after lenticular implantation. The preoperative corrected distance visual acuity improved from  $0.86 \pm 0.22$  logMAR to  $0.47 \pm 0.09$  logMAR ( $P < .001$ ). The preoperative central corneal thickness increased from  $399 \pm 13.29$   $\mu\text{m}$  to  $482.10 \pm 8.12$   $\mu\text{m}$  ( $P < .001$ ).

**Conclusion:** Stromal regeneration seems possible in patients with KCN with stem cells and telocytes.

**ClinicalTrials.gov identifier:** NCT04591587.

## Rose Bengal Photodynamic Therapy Safety Study

Jaime Martinez Martinez<sup>1</sup>, Guillermo Amescua<sup>1</sup>, Sander Dubovy<sup>2</sup>, Roger Leblanc<sup>1</sup>, Jean-Marie Parel<sup>1</sup>

1. Bascom Palmer Eye Institute, Miami, Florida, USA

2. Bascom Palmer Eye Institute

Corresponding author: Dr Jaime Martinez Martinez, Bascom Palmer Eye Institute, Miami, Florida, USA, [jaimedavidmartinez@gmail.com](mailto:jaimedavidmartinez@gmail.com)

**Purpose:** To evaluate corneal changes in healthy rabbits after Rose Bengal Photodynamic Therapy treatment (PDT) and different concentrations of Rose Bengal diluted strips.

**Methods:** Eighteen rabbits were divided into five groups, each with three rabbits, except group 5, with six rabbits. Group 1: corneal de-epithelization (CDE), Group 2: CDE and Rose Bengal drops (8 strips of 1.3mg in 5cc BSS) every 3 minutes for 30 minutes, Group 3: CDE and Green light exposure 5.4 J/cm<sup>2</sup> for 15 minutes. Group 4, four strips in 5 cc and PDT, and Group 5, eight strips in 5 cc and PDT. Rose Bengal (RB) solution analysis was performed: Four sterile RB strips and eight RB strips were diluted at different time points in 5 ccs of balanced salt solution (BSS).

**Results:** Epithelium defect healed on day 3 in groups 1-4. In group 5, 3 out of 6 rabbits had persistent epithelium defects. Rose Bengal concentration for four RB strips in 5 cc BSS as measured at 1 minute was  $0.0965 \pm 0.001\%$ , at 2.5 minutes was  $0.134 \pm 0.001\%$ , 5 minutes  $0.154 \pm 0.002\%$ , and 10 minutes  $0.176 \pm 0.004\%$ . Rose Bengal concentration for eight RB strips in 5 cc BSS as measured at 10 min was  $0.310 \pm 0.008\%$ , at 70 min was  $0.340 \pm 0.015\%$ , and at 130 min was  $0.36 \pm 0.01\%$ .

**Conclusion:** Rose Bengal photodynamic therapy using 4 Rose Bengal strips is a safe procedure with the same epithelial healing time as the control group compared to 8 strips RB group.