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Identifying, Characterising and Modifying the Natural History and Progression of Keratoconus in New Zealand/ Aotearoa

Charlotte Ann Jordan

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy, Department of Ophthalmology The University of Auckland, 2012.
Keratoconus, the focus of this thesis, is a progressive ectasia (thinning/bowing) of the cornea thought to be more prevalent in New Zealand with a predilection for Maori and Pacific populations. Keratoconus occurs due to a combination of genetic and environmental factors. Typically, diagnosis of keratoconus is made on the basis of clinical and corneal topographic/tomographic signs.

A large population of advanced keratoconics was analysed for tomographic and phenotypic variations between subjects with differing aetiological risk factors. This study specifically identified phenotypic differences occurring between subjects with, and without, a family history. The results confirmed over-representation of Maori and Pacific ethnicities in the New Zealand keratoconic population and identified largely asymmetric corneal disease by tomographic classification.

The Ocular Response Analyser (ORA) was employed to investigate the intrinsic biomechanical properties of the normal and keratoconic cornea. Significant correlations were observed between posterior corneal elevation and corneal resistance factor in the keratoconic cohort. However, no single ORA value was identified as a discriminator of keratoconus, nonetheless, combining these factors may increase their diagnostic sensitivity.

Corneal collagen cross-linking aims to halt, or slow, the progression of keratoconus. This novel therapy involves utilising ultra violet light (UVA) and the photosensitiser riboflavin to stimulate formation of covalent bonds between corneal collagen fibrils. This improves the mechanical rigidity of the cornea and increases resistance to the ectatic process. In a large randomised controlled trial (RCT) of collagen cross-linking for keratoconus in New Zealand, corneal keratometry reduced (improved) in the majority of treated eyes, while visual acuity
and refraction remained stable. In contrast, control, untreated, contralateral eyes showed continued progression in both keratometric and refractive indices.

A unique quantitative study by in vivo confocal microscopy revealed significant reduction in the sub-basal nerve plexus and anterior keratocyte density following cross-linking in keratoconus. These effects persisted over 12 months post-operatively. Dense hyper-reflective bands developed in the corneal mid-stroma following treatment that reduced in intensity over 24 months post cross-linking.

These inter-related studies provide new data on keratoconus in New Zealand, on the application of diagnostic techniques, and the safety and effectiveness of collagen cross-linking for keratoconus in a large RCT.
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Table 8-19: Mean corneal hysteresis compared to pre-operative values at the 1, 3, 6 and 12 months follow up reviews. Statistical values in columns two and three compares the mean values to the pre-operative values at each review time. Statistical values in column five compare the mean change in corneal hysteresis between treated and control (untreated) eyes at each time point.

Table 8-20: Mean corneal resistance factor in treated and control eyes compared to pre-operative values at the 1, 3, 6, 12 and 24 months follow up reviews. Statistical values in columns two and three compares the mean values to the pre-operative values at each review time. Statistical values in column four compare the mean change in the corneal resistance factor between treated and control (untreated) eyes at each time point.

Table 9-1: Published human clinical studies of corneal collagen cross-linking in the treatment of keratoconus presented in chronological order with summary of key observations.
# LIST OF ABBREVIATIONS USED IN THIS THESIS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>µm</td>
<td>Microns (micrometres)</td>
</tr>
<tr>
<td>AER</td>
<td>Anterior Elevation Ratio</td>
</tr>
<tr>
<td>AGEs</td>
<td>Advanced Glycation End products</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>AS-OCT</td>
<td>Anterior Segment Optical Coherence Tomography</td>
</tr>
<tr>
<td>AST</td>
<td>The Astigmatism Index</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>BCVA</td>
<td>Best-Corrected Visual Acuity</td>
</tr>
<tr>
<td>BFS</td>
<td>Best Fit Sphere</td>
</tr>
<tr>
<td>CCLRU</td>
<td>Cornea and Contact Lens Research Unit</td>
</tr>
<tr>
<td>CCT</td>
<td>Central Corneal Thickness</td>
</tr>
<tr>
<td>CH</td>
<td>Corneal Hysteresis</td>
</tr>
<tr>
<td>CLEK</td>
<td>Collaborative Longitudinal Evaluation of Keratoconus</td>
</tr>
<tr>
<td>CRF</td>
<td>Corneal Resistance Factor</td>
</tr>
<tr>
<td>D</td>
<td>Diopters</td>
</tr>
<tr>
<td>DALK</td>
<td>Deep Anterior Lamellar Keratoplasty</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
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<tr>
<td>DUSKS</td>
<td>Dundee University Scottish Keratoconus Study</td>
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<tr>
<td>FFKCN</td>
<td>Forme Fruste Keratoconus</td>
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<tr>
<td>FH</td>
<td>Family History</td>
</tr>
<tr>
<td>g/mol</td>
<td>grams per mole</td>
</tr>
<tr>
<td>GAG</td>
<td>Glycosaminoglycan</td>
</tr>
<tr>
<td>ICC</td>
<td>Intra-class Correlation Coefficient</td>
</tr>
<tr>
<td>IL-1</td>
<td>Interleukin-1</td>
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ICRS  Intra-corneal Ring Segments
IOP  Intra-Ocular Pressure
IOP<sub>cc</sub>  Corneal Compensated Intra-Ocular Pressure
IOP<sub>G</sub>  Goldmann Correlated Intra-Ocular Pressure
I-S Value  Inferior - Superior Value
IVCM  In-vivo Confocal Microscopy
J/cm<sup>2</sup> Joules per centimetre squared
K  Keratometry
KISA%  Keratoconus Percentage Index - a product of four indices
LASIK  Laser In Situ Keratomileusis
LED  Light Emitting Diode
LogMAR  Logarithm of the Minimum Angle of Resolution
MaxK  Maximum Keratometry
MinK  Minimum Keratometry
mm  Millimetres
mmHg  Millimetres of Mercury
MPO  Myeloperoxidase
mW/cm<sup>2</sup> Milliwatt per Centimetre squared
Na  Sodium
Nerve/mm<sup>2</sup> Nerve per millimetre squared
nm  Nanometres
ORA  Ocular Response Analyser
P1  Applanation Pressure 1
P2  Applanation Pressure 2
PER  Posterior Elevation Ratio
PKP  Penetrating Keratoplasty
<table>
<thead>
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<th>Abbreviation</th>
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<tr>
<td>PRK</td>
<td>Photorefractive Keratectomy</td>
</tr>
<tr>
<td>RCM</td>
<td>Rodenstock Corneal Module</td>
</tr>
<tr>
<td>RGP</td>
<td>Rigid Gas Permeable</td>
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<tr>
<td>RMS</td>
<td>Root Mean Squared</td>
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<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
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<tr>
<td>SAS</td>
<td>Statistical Analysis System</td>
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<tr>
<td>SD</td>
<td>Standard Deviation</td>
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<tr>
<td>SimK</td>
<td>Simulated Keratometry</td>
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<tr>
<td>SRAX</td>
<td>Skewed Radial Axes</td>
</tr>
<tr>
<td>TUNEL</td>
<td>Terminal Deoxynucleotidyl Transferase dUTP Nick End Labelling</td>
</tr>
<tr>
<td>UCVA</td>
<td>Un-Corrected Visual Acuity</td>
</tr>
<tr>
<td>US</td>
<td>Ultrasound</td>
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<tr>
<td>UV</td>
<td>Ultraviolet</td>
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<td>UVA</td>
<td>Ultraviolet A</td>
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SECTION 1

Introduction and Examination Techniques

Chapters 1-3
Chapter 1 : Keratoconus
Chapter 1

1.1 INTRODUCTION

Keratoconus is a non-inflammatory, progressive ectasia of the cornea that usually presents in early puberty, and progresses until the third to fourth decade of life when the disease tends to stabilize, although the progression can be variable throughout a patient’s life. ¹

Internationally, keratoconus has a variably reported prevalence of between 50 and 230 per 100,000. ¹-³ An increased prevalence of this disease has long been postulated in the New Zealand population, possibly reflected in a high national rate of corneal transplantation for keratoconus compared with international data, ⁴ ⁵ though this might also reflect greater severity of disease. Keratoconus has been reported with incidence of 1 in 2000 in regions of New Zealand, ⁶ and reports have suggested that Maori and Pacific populations are over represented in the New Zealand Keratoconic populations. ⁷

Regions such as the Asir Province of Saudi Arabia have also reported a high incidence of keratoconus. ⁸ United Kingdom based studies have revealed rates of keratoconus in people of Asian descent (most commonly from Northern Pakistan) born in the UK to be as high as 25 per 100 000 (1 in 4000) per year, compared with only 3.3 per 100 000 (1 in 30 000) per year for local white caucasian populations. These data suggest that ethnic background significantly influences the incidence and the severity of keratoconus, since the Asian subjects present at a significantly younger age than caucasian subjects and also appeared to suffer a more severe form of the ectatic disease. ⁹ ¹⁰
1.2 THESIS AIMS

1. Computerised Corneal Tomography and Associated Features in a Large New Zealand Keratoconic Population

The aim of this study was two-fold; to investigate the tomographic features of keratoconus and to explore associations between keratoconus risk factors and disease phenotype in a large hospital-based population with keratoconus. The study hypothesis being: advanced keratoconus in the New Zealand population may exhibit unique associations between known risk factors for the disease and phenotypic expression.

2. Repeatability and Variability of Intraocular Pressure and Corneal Biomechanical Properties as Measured by the Ocular Response Analyser

The aims of this ORA based study were a) to evaluate the diurnal variation in IOP and corneal biomechanical properties b) to evaluate the day to day variation in IOP and corneal biomechanical properties c) to determine any relationship between diurnal variation of IOP, corneal hysteresis (CH) and corneal resistance factor (CRF), d) to assess the intraobserver repeatability of ORA measurements. The study hypothesis being: ORA measures of corneal biomechanical properties and IOP measures are reproducible and uninfluenced by diurnal, day-day or observer variables.

3. Corneal Hysteresis and Corneal Resistance Factor in Keratoconus

The aim of this study was to determine the relationship between ORA measured corneal biomechanical properties - specifically Corneal Resistance Factor (CRF) and Corneal
Hysteresis (CH) - and tomography measured posterior corneal elevation. The study hypothesis being: corneal biomechanical properties measured by the ORA are influenced by the tomographic properties of the keratoconic cornea.

4. Corneal Collagen Cross-linking in a New Zealand Population

The aim of this study was to investigate the efficacy of corneal collagen cross-linking in halting the progression of ectasia in keratoconus. The efficacy of cross-linking was investigated using a randomised control trial with the untreated eye of each subject acting as a control. The study hypothesis being: progression of keratoconus ectasia in collagen cross linking treated eyes is halted or greatly reduced, whereas, untreated control eyes continue to progress.

1.3 AETIOLOGY

Several aetiological associations have been postulated with keratoconus, including, family history, atopy and eye rubbing. \(^{11,12}\) Interestingly, a family history of keratoconus has been reported in only 6% to 20% of affected individuals, \(^2,13-16\) although an autosomal dominant mode of inheritance is postulated. However, many researchers concur that the disease aetiology is multifactorial with both genetic and environmental elements coupled with variable expressivity. \(^3,17-19\)

The association between keratoconus and atopy is long established, although whether this is a coincidental, or causal, relationship is still debated. Atopy includes conditions such as eczema, asthma, and hayfever. Many studies have reported increased rates of atopic eye disease in keratoconic subjects compared to the unaffected population. \(^11,15,20-23\)
study examined 1206 keratoconic subjects across the United States and noted that 52.9% of subjects had hayfever or allergies, 14.9% had asthma, and 8.4% had atopic dermatitis. High self-reported rates of asthma and hayfever were also noted in patients with keratoconus presenting to Moorfields Eye Hospital, London, with 32.6% having hayfever and 33.3% reporting asthma. The Dundee University Scottish Keratoconus Study (DUSKS) noted self-reported rates of asthma, eczema and hayfever of 23%, 14% and 30% respectively. The prevalence of asthma, eczema and systemic allergy in New Zealand has been reported as 22.2%, 15%, and 11.4% respectively. Harrison et al noted that all bar one of 76 keratoconic patients had developed keratoconus after the onset of atopy.

Many multi-system disease associations with keratoconus have been reported including Down syndrome, Marfan syndrome and Ehlers-Danlos syndrome. Ocular disease associations include floppy eyelid syndrome, retinitis pigmentosa and vernal keratoconjunctivitis.

Eye-rubbing has long been postulated as a contributing factor to keratoconus. The association between eye rubbing and allergic eye disease led to the theory that long term mechanical trauma, due to chronic digital stimulus, contributes to the eye disease. It has also been hypothesized that the cone is typically located in the infero-nasal quadrant because of the chronic trauma of eyelid rubbing and epithelial damage in this area. It has also been postulated that the oldest epithelial cells are located in this area, and subsequently may be more vulnerable to mechanical damage than younger cells. In the CLEK study, 48.2% of keratoconic patients reported rubbing both eyes vigorously, whereas only 2.2% reported rubbing only one eye vigorously. Another study reported that the more severely affected eye often corresponds to the side of the dominant hand.
Predictors for the severity of keratoconus were investigated by Szczotka-Flynn et al. in conjunction with the CLEK study. Using univariate analyses, none of the severity indices (corneal scarring, mean corneal power, flat keratometry value, steep keratometry value, and higher order first corneal surface wavefront RMS error) were significantly associated with a family history of the disease. Interestingly, Barr et al, using the CLEK study sample but a different methodology, did not find family history to be predictive of corneal scarring. The CLEK study also reported that patients with more severe disease were more asymmetric in their disease status, and more advanced disease was associated with a greater likelihood of the presence of Vogt’s striae, Fleischer ring and/or corneal scarring.

A study investigating clinical associations reported a significant association between keratoconus and atopy as well as eye rubbing and family history of keratoconus when univariate analysis was performed, however, only eye rubbing was a significant predictor of keratoconus when multivariate analysis was performed. These observations raise the possibility that atopic patients who develop keratoconus may do so because of the eye rubbing behaviour that atopic individuals develop as a consequence of itchy eyes.

1.4 NORMAL CORNEAL ANATOMY AND PHYSIOLOGY

The cornea together with the sclera forms the dense outer shell of the eyeball. The vascular limbus constitutes the transitional zone between the cornea and sclera. The cornea measures 11 to 12mm horizontally and 9 to 10mm vertically. It is approximately 0.5mm thick at its centre this increases to 0.7mm thick at its periphery (Figure 1-1). The radius of curvature is greatest measuring between 7.5 to 8mm at the central 3mm optical zone. In this area the corneal surface is almost spherical. The radius of curvature is least at the periphery. At the limbus because of the difference in curvature between the cornea and sclera a slight sulcus is seen, the sulcus sclerae.
Chapter 1

Figure 1-1: Anterior segment Optical Coherence Tomography showing the corneal and anterior chamber dimensions with a normal corneal curvature and thickness.

The cornea constitutes about two-thirds of the refractive power of the eye; this measures approximately 40 to 44 dioptres. The optical properties of the cornea are determined by its transparency, smooth surface, radius of curvature and refractive index (1.376). 35

The long ciliary nerves provide the sensory innervation of the cornea. They are derived from the ophthalmic branches of the trigeminal nerves. 36 The density of nerve endings in the cornea is 300 to 400 times greater than that of the skin. The long ciliary nerves penetrate the deep peripheral stroma radially, losing myelination as they enter the corneal stroma (Figure 1-2A). The nerves track anteriorly forming a sub-epithelial and a sub-basal nerve plexus with termination at the level of the wing cells in the corneal epithelium (Figure 1-2B). 35 37 38
**Figure 1-2**: A Diagram indicating the nerve supply to the cornea showing the long ciliary nerves penetrating the deep peripheral stroma radially where the nerves move anteriorly forming a terminal sub-epithelial plexus. B. Diagram of a corneal section showing the corneal nerves moving through the stroma, penetrating Bowman’s layer and entering the epithelium. (Images MD, non-copyright)

The cornea is an avascular tissue and whilst the normal cornea does not contain blood vessels, haematogenous factors required for healing and corneal metabolisms are necessary. These are derived from the limbal vasculature arcade that is served by the anterior ciliary arteries that travel via the recti muscles and also by the vessels derived from the facial branch of the external carotid artery. A supply of glucose and oxygen are essential for the normal metabolic function of the cornea. Glucose is supplied by diffusion from the aqueous humour whilst oxygen is delivered primarily by diffusion from the tear film which absorbs oxygen from the air, thus closure of the lids during sleep and contact lens wear reduces the amount of oxygen that reaches the cornea.

Microscopically the cornea consists of five layers: the epithelium, Bowman’s layer, corneal stroma, Descemet’s membrane and the corneal endothelium (Figure 1-3). Overlying the epithelium is the pre-corneal tear film.
Figure 1-3: Haematoxylin and Eosin stain of the Corneal Layers

1.4.1 Corneal Tear Film

The tear film protects the cornea from dehydration as well as maintaining a smooth optical and epithelial surface. The pre-corneal tear film has been reported to measure between 7-50μm in thickness and consists of three layers; a superficial mucus layer approximately 0.1μm thick, an aqueous layer 7μm thick and a mucus layer adjacent to the corneal epithelium (0.05μm). The tear film not only serves as a surface lubricant but because of its high concentration of active substances (secreted predominantly from the lacrimal and accessory lacrimal glands) such as growth factors, cytokines and prostaglandins, provides factors required for maintenance of epithelial integrity, growth and repair.35, 40
1.4.2 Corneal Epithelium

The corneal epithelium provides an important barrier function to external stimuli. The presence of junction complexes between adjacent cells prevents the passage of agents into the deeper corneal tissue. Tight junctions (zona occludens) exist predominantly between the superficial cell layers. Hemidesmosomes (zona adherens) and desmosomes are present in all layers. Gap junctions (which allow passage of small molecules) are present in the wing and basal cell layers.\(^{35}\)

The corneal epithelium consists of non-keratinised, stratified, squamous epithelial cells.\(^{35}\) The corneal epithelium is continuous with the conjunctival epithelium. The epithelium is approximately 50\(\mu\)m thick and consists of five or six layers of three different epithelial cells:

1. Two or three layers of terminally differentiated flat and polygonal superficial cells. The surfaces of these cells are covered with microvilli that in addition to the hydrophilic properties of the superficial cells aid in tear film mucin adhesion.\(^{35}\)
2. Two or three layers of wing cells. These cells represent an intermediate stage of differentiation between the basal and superficial cells.\(^{35}\)
3. A single layer of columnar basal cells. Basal cells are the only corneal epithelial cells that undergo mitosis, differentiating into wing cells then subsequently superficial cells. This process of differentiation takes seven to fourteen days. Following this superficial cells are desquamated into the tear film. The corneal epithelium is therefore in a constant stage of regeneration. Basal cells adhere to the basement membrane via hemidesmosomes, which are in turn attached by type VII collagen fibrils through to the corneal stroma where they form anchoring plaques with type I collagen.\(^{35}\)
1.4.3 Bowman’s Layer

Bowman’s layer is an acellular membrane-like layer consisting of randomly arranged type I and III collagen. It measures approximately 10\(\mu\)m thick and does not regenerate after trauma.\(^{35}\) The collagen fibres in Bowman’s layer are secreted by stromal keratocytes. The exact physiological function of Bowman’s layer in relation to the epithelium is still unknown. This is demonstrated following excimer laser photoablation of Bowman’s layer, as normal corneal epithelium continues to be formed even with its absence.\(^{35}\)

1.4.4 Corneal Stroma

The stroma constitutes more than 90% of the corneal thickness. Corneal transparency, stability and strength are due to the anatomical and biochemical properties of the stroma.\(^{41}\) The stroma connects the anterior sclera at the limbus, the tissue here loses its transparency because of the non-uniform arrangement of the collagen fibrils. Keratocytes occupy 2-3% of the volume of the corneal stroma and are the main cellular component. These spindle shaped cells are thought to turn over every 2 to 3 years.\(^{35}\)

In response to insults to the cornea, keratocytes are activated and undergo transformation into myofibroblasts that express smooth muscle actin.\(^{35, 42}\) Myofibroblasts produce extracellular matrix, matrix-metalloproteinases and cytokines for stromal tissue repair. Myofibroblasts have contractile ability, which also contributes to their wound healing capabilities.\(^{42}\)

The remainder of the cornea stroma comprises of collagen fibrils (collagen constitutes 70% of the dry weight of the cornea) and proteoglycans that together constitute the extracellular matrix. The predominant collagen type in the stroma is type I (there are smaller amounts
of type III, V and VI). The diameter of the collagen fibres (22.5-35nm) and the distance between them (41.4± 0.5nm) is roughly half the wavelength of visible light (400-700μm). It is thought that destructive interference from scattered incident light rays is responsible for corneal transparency (Figure 1-4).

![Electron micrograph (X32 000) of the posterior stroma showing alternating cross-sectioned and longitudinal sectioned collagen bundles and Keratocytes (K).](image)

**Figure 1-4:** Electron micrograph (X32 000) of the posterior stroma showing alternating cross-sectioned and longitudinal sectioned collagen bundles and Keratocytes (K).

The remainder of the extracellular matrix consists of proteoglycans. These are thought to modulate collagen fibrillogenesis and play a role in corneal hydration. Proteoglycans consist of a protein core (lumican, keratocan, mimecin and decorin) and a glycosaminoglycan chain (GAG). Keratan sulphate is the most prevalent GAG in the stoma constituting 65% of GAG content. Remaining GAGs include chondroitin and dermatan sulphate.
1.4.5 Descemet’s Membrane

Descemet’s membrane acts as the basement membrane for the corneal endothelium. It consists of type IV and VIII collagen, laminin and fibronectin. The membrane is tightly adhered to the posterior surface of the corneal stroma. It can be torn or ruptured by physical stress such as that seen in corneal hydrops resulting in aqueous flow into the corneal stroma and stromal oedema.

1.4.6 Corneal Endothelium

The corneal endothelium constitutes a single layer of cells that is 5μm thick. At birth there are approximately 4000 cells/ mm$^2$. Corneal endothelial cells do not proliferate and their number therefore decreases with the attrition of age - the density reducing to 3500 cells/ mm$^2$ or less in young adulthood. The cells are typically hexagonal and uniform in shape. Deviation from this uniformity occurs with cell damage where loss of cells results in enlargement (polymegethism) and spreading of the adjacent cells to cover the defect resulting in irregular cellular shapes (pleomorphism).

The corneal endothelium is metabolically very active and is primarily responsible for regulation of corneal hydration via Na$^+$/ K$^+$ ATPase driven osmotic pump. Corneal oedema occurs at an endothelial density of approximately 500 cells/ mm$^2$, as the compensatory activity of the endothelial pumps is insufficient to maintain optimum corneal hydration.
1.5 PATHOPHYSIOLOGY

1.5.1 Corneal Epithelium

Evidence from histopathological studies conclude that the earliest pathologic changes in keratoconus occur in the basal layer of the corneal epithelium. Proteolytic enzymes released by these degenerating cells cause fragmentation of the basement membrane, fibrillation of Bowman’s layer, and loss of stromal collagen fibrils.\textsuperscript{48}

\textit{In vivo} confocal microscopy studies of the corneal epithelium in keratoconus have noted visibly elongated superficial epithelial cells arranged in a whorl pattern at the corneal apex. Basal epithelial cell density is also reduced in keratoconic corneas when compared to normal corneas, with the density negatively correlated with the severity of the disease.\textsuperscript{49}

The disruption of the basal epithelium that occurs in keratoconus has been further explained by identification of apoptotic markers (TUNEL positive) in the deeper levels of a keratoconic epithelium in ex vivo studies. In contrast, in normal healthy corneas TUNEL-positive epithelial cells were only detectable in the superficial epithelium\textsuperscript{50}

1.5.2 Bowman’s Layer

Early degeneration of the basal epithelial cells is followed by disruption of the corneal epithelial basement membrane. Subsequent breaks in Bowman’s layer may be accompanied by focal down growth of the epithelium through Bowman’s layer into the anterior stroma.\textsuperscript{48}
Structural abnormalities and defects in Bowman’s layer at the apex of keratoconic corneas have been well documented histopathological and ultra-structural studies have revealed disintegration of Bowman’s layer at early stages of the disease. Sawaguchi et al. noted multiple sharp edged defects in Bowman’s layer in all eight corneal buttons obtained from patients with the typical clinical features of keratoconus. A lattice-like configuration of perforated Bowman’s layer, was associated with hypertropic collagenous scar tissue formation within the perforations.

Two histopathological variants of keratoconus were identified by Scroggs et al. in a study observing changes in corneal specimens obtained following penetrating keratoplasty. The typical keratoconic specimen exhibited central corneal epithelial thinning with multiple breaks in Bowman’s layer. In the atypical variant, there were no breaks in Bowman’s layer and there was less thinning in the central corneal epithelium.

1.5.3 Corneal Nerves

Prominent corneal stromal nerves are commonly visible (clinically) in keratoconus, and a number of studies have therefore investigated the involvement of nerve fibres in keratoconus. Thickened nerve fibre bundles passing between the stroma and epithelium have been noted to closely align with abnormalities in Bowman’s layer. Recent studies have also noted decreased sub basal nerve plexus density in keratoconic corneas when compared to normal, with a negative correlation in terms of density with a younger age of diagnosis, a history of eye rubbing and a Maori or Pacific Island ethnicity.
1.5.4 Corneal Stroma

In the healthy cornea, between 80% and 90% of corneal stromal collagen fibres are type I collagen. Type III and type V collagen are also present in the stroma. Type IV collagen is present in Descemet’s membrane and the corneal epithelial basement membrane. As previously noted the non-fibrillar extracellular matrix is primarily composed of glycosaminoglycan proteoglycans. These are acid mucopolysaccharides attached to a relatively small protein core. The glycosaminoglycans (GAGs) most prevalent in the normal cornea are keratan sulfate (approximately 65%) and chondroitin-4-sulfate (approximately 30%). Proteoglycans play an important role in regulating collagen fibril growth and diameter and therefore play a key role in the maintenance of corneal transparency. Their presence also influences corneal hydration.

Interestingly, transmission electron microscopy studies of diseased tissue have revealed that the thickness of collagen lamellae is unaltered in keratoconus, whereas, the total number of lamellae appears to be significantly less than in the normal cornea. This combined with a reduced number of natural cross-links between the collagen fibres in keratoconus and alteration of the regular orthogonal arrangement of the collagen fibrils may be responsible for the biomechanical instability of the tissue.

In an attempt to explain the stromal degradation observed in this non-inflammatory disease, multiple theories have been proposed. These include increased expression of lysosomal enzymes, decreased activity of the inhibitors of proteolytic enzymes, and damage mediated by Interleukin-1 (IL-1) in subjects with keratoconus who rub their eyes. Oxidative damage has also been postulated to be a component in the pathogenesis, with keratoconic corneas being less able to neutralise reactive oxidative species generated by environmental and mechanical injury.
Apoptosis has been shown to occur in keratoconic corneas, with evidence of TUNEL positive cells in the cornea epithelium, stroma and endothelium. Chronic keratocyte apoptosis associated with on-going epithelial injury may link risk factors associated with keratoconus such as chronic eye rubbing, contact lens wear, or atopic eye disease. It has been hypothesized that chronic triggers of keratocyte apoptosis may lead to changes in the cornea that include a decrease in the total number of keratocytes, release of degradative enzymes, and subsequent loss of corneal stroma over a period of time. A study investigating anterior stromal keratocyte changes following epithelial scrape wounding noted cell shrinkage, blebbing with formation of membrane bound bodies, condensation and fragmentation of the chromatin, and DNA fragmentation, consistent with apoptosis. The authors concluded that the disappearance of keratocytes from the underlying stroma following epithelial debridement is mediated by apoptosis initiated by interleukins.

Fabre et al noted that fibroblasts in keratoconic corneas have four times more IL-1 binding sites than normal fibroblasts. The authors suggested that IL-1 stimulates synthesis of collagenase and prostaglandins by fibroblasts, and that the increase of IL-1 binding site numbers on these cells may be responsible for the reduction of collagen in keratoconus. Apoptosis of stromal keratocytes has also been reported following the epithelial injury induced during refractive surgery such as photorefractive keratectomy (PRK) and laser in situ keratomileusis (LASIK).
1.6 DIAGNOSTIC CRITERIA

1.6.1 Clinical Signs of Keratoconus: Slit Lamp Biomicroscopy

The diagnosis of keratoconus is typically made on the basis of a combination of clinical signs and corneal topographic/tomographic signs (Figure 1-6). Early clinical signs include progressive refractive and topographic astigmatism. Classic clinical signs on slit lamp biomicroscopy include; corneal thinning, conical protrusion, (Figure 1-5) (Figure 1-6) Fleisher’s ring, Vogt’s striae, enlarged/prominent corneal stromal nerves and Rizzuti’s sign. Other signs include the scissoring reflex on retinoscopy and the oil droplet sign visible with direct ophthalmoscopy. In the more severe stages of the disease, signs may include corneal scarring (epithelial or subepithelial), Munson’s sign (V-shaped conformation of the lower lid upon down-gaze) and in more extreme cases, acute corneal hydrops (due to rupture in Descemet’s membrane) (Figure 1-6).

Figure 1-5: A. Optic section of inferior conical protrusion of the cornea in keratoconus (indicated by arrow) B. Optical section of a normal cornea (indicated by arrow).
Subjects with none of the aforementioned clinical findings and good best-corrected visual acuity, but who exhibit abnormal corneal topography, may have Forme Fruste keratoconus (FFKCN). Many different titles have been suggested for early stage or subclinical keratoconus including keratoconus suspect and forme fruste keratoconus. Forme fruste by definition is “an incomplete, abortive, or unusual form of a syndrome or disease” and would therefore describe the non-progressive form of keratoconus. Sub-clinical and keratoconus suspect, may therefore be considered to describe early disease, prior to any confirmation of progression or non-progression (stability/FFKCN).
**Figure 1-6:** Key clinical signs of keratoconus.

**A,B.** highlight corneal thinning and conical protrusion visible in an optical section in keratoconus (Indicated by arrows).

**C,D.** Fleisher’s ring, a partial or complete iron deposition ring in deep epithelium encircling the base of the cone, is visible (indicated by arrows) using **C.** a cobalt blue filter and **D.** white light.

**E.** Vogt’s vertical striae are visible at the corneal apex of a keratoconic cornea (Indicated by arrow).

**F,G.** Show prominent corneal nerves indicated by arrows (**F.** 20x, **G.** 40x magnification).

**H.** Munson’s sign (conical protrusion noted on down gaze).

**I,J.** Acute Corneal hydrops due to rupture in Descemet’s membrane (**I.** 20x, **J.** 10X magnification).
Progression of refractive error is often the earliest sign of keratoconus, with progressive irregular astigmatism with or without a myopic shift. As the disease progresses, more complex spectacle prescriptions are required and eventually rigid contact lenses may be required to provide functional vision. The CLEK study noted that over 8 years the mean rate of progression the flattest keratometric value was 0.2 ± 0.8 D/year, which can be extrapolated to a mean progression rate of 1.60D in the flattest corneal curvature over their 8-year follow up period. However, the authors also noted that flattest corneal curvature increased by 3.0 D or more in at least one eye in 24% of subjects. The 8-year incidence of penetrating keratoplasty was higher among patients aged less than 40 years old (15%) compared to patients 40 years of age or older (8%).

Keratoconus is largely regarded as a bilateral but asymmetric disease, affecting one eye earlier and more severely in most cases. Studies of inter-eye asymmetry in corneal curvatures have noted substantial differences of 3.5 - 4.0D between eyes. It has also been reported that patients with more severe disease have greater asymmetry in steep and flat keratometric readings and refractive error between their eyes.

1.5.2 Corneal Topography and Tomography in Keratoconus

Prior to the introduction of computer assisted tools, the diagnosis of keratoconus relied on hand held keratoscopes incorporating Placido discs (Figure 1-7). The diagnostic criteria included a downward deviation of the horizontal axis of the Placido disk reflection.

The traditional keratometer, either one or two position, provided information about only 2 or 4 points approximately 3 mm apart (Figure 1-7). These detected keratoconus by showing distortion of the mires or central or inferior corneal steepening. However, examination of the para-central cornea in keratoconic eyes requires alteration of the standard technique,
as the ectasia is often away from the central axis which the keratometer measures. Another limitation of early keratometers was that the degree of steepening in keratoconic corneas often exceeded the standard upper limit of the instrument, so accurate measurements could not be ascertained.

Figure 1-7: A. The Bausch and Lomb (Bausch and Lomb, Rochester, NY, USA) one position keratometer B. A hand-held Placido disc.

1.5.3 Computer-assisted Corneal Topography/ Tomography

With advances in corneal refractive surgery and the development of clinical practices such as contact lens fitting that are reliant on accurate corneal curvature measurements, computerised videokeratography was introduced. In the early stages the instruments solely incorporated the early, well-established, Placido disc technology, and later included newer techniques such as slit beam scanning and Scheimpflug imaging (Figure 1-9) (See Chapter 2.3). Computerised videokeratography is now a well-established tool for diagnosing and monitoring keratoconus. 1,74,75
Li et al recently used a combination of computerised videokeratography data and clinical signs to produce a practical classification system for the identification of early keratoconus. The authors noted that when only the central keratometric value was assessed, 36.2% of keratoconus suspects were diagnosed correctly and 34.9% of mild keratoconics were diagnosed correctly. The KISA% (keratoconus percentage index—a product of four indices) uses minimal topographic data yet is noted to have the highest rate of correct diagnosis for mild keratoconus, with a correct classification rate of 78.8%. The KISA% index combines central K, I-S (Inferior- superior keratometry), the astigmatism index (AST), which quantifies the degree of the regular corneal astigmatism, (simulated K1 - simulated K2); and the SRAX (skewed radial axes) index. A combination of Central K, I-S, and KISA% and age of the patient was reported to have the highest rate of diagnosing suspect and mild keratoconus, with correct classification rates of 44.6% and 75.3% respectively.

**Figure 1-8:** Standard quad-map in mild keratoconus measured with the Orbscan II tomographer (Bausch and Lomb, Rochester, NY, USA).
The computerised topographic features of keratoconus are outlined in Table 1, however, as outlined in the preceding studies, no single parameter can be used in isolation to correctly identify keratoconus with 100% accuracy. However, several reports have identified practical topographic parameters important in distinguishing keratoconus from normal corneas, these include: central corneal keratometric steepening and inferior-superior dioptric asymmetry. Other significant, but less commonly used parameters, identified as being statistically different in keratoconus include corneal volume, and anterior chamber angle and depth.

Anterior and posterior corneal surface elevation data measured by Orbscan II (Figure 1-8) and Pentacam has proved useful in distinguishing between normal and keratoconic corneas, and in the early detection of keratoconus. Fam and Lim noted that the anterior elevation ratio (anterior elevation/anterior best fit sphere) had the highest sensitivity for diagnosing keratoconus followed by anterior elevation, posterior elevation, posterior elevation ratio (posterior elevation/posterior best fit sphere), Kmax, and Kmin using the Orbscan II system.

Studies using Pentacam rotating Scheimpflug tomography, have shown a high sensitivity and specificity when a posterior surface elevation cut-off point of 35 µm is used for keratoconus and 29 µm is used for subclinical keratoconus. Other studies have suggested a cut-off point of less than 40µm for refractive surgery candidates to exclude corneas with possible keratoconic changes. A posterior elevation of 40 µm was selected as a screening limit because it was approximately 2 standard deviations greater than the mean posterior elevation for the control group examined in the study. However other studies have suggested that posterior elevation is less sensitive than anterior elevation and that using a cut off elevation of 40 µm or more had a sensitivity of 57.7% and specificity of 89.8% in distinguishing keratoconus and keratoconus suspects from normal corneas.
<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>VIDEO-KERATOGRAPHY</th>
<th>ORBSCAN</th>
<th>PENTACAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-S Value (Inferior- Superior Value) (D)</td>
<td>&gt;1.20 (^{84,85})</td>
<td></td>
<td>&gt;1.20 ± 0.92 (^{84,86})</td>
</tr>
<tr>
<td></td>
<td>&gt;1.40 (^{85})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simulated Keratometric astigmatism (D)</td>
<td>&gt;1.5 (^{84})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRAX (Skewed Radial axes) (degrees)</td>
<td>&gt;21 deg (^{84,85})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Keratometry (D)</td>
<td>&gt;47.2 (^{84})</td>
<td>&gt;45.09 (^{88})</td>
<td>&gt;44.35 (^{89})</td>
</tr>
<tr>
<td></td>
<td>&gt;45.13 (^{86,87})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior elevation (µm)</td>
<td>&gt;40 (^{82})</td>
<td>&gt;35 (^{81})</td>
<td>&gt;40 (^{90})</td>
</tr>
<tr>
<td></td>
<td>&gt;46 (^{87})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corneal Volume (10mm Diameter disc) (mm(^3))</td>
<td></td>
<td>57.3 ± 2.12 (^{88})</td>
<td>57.8 (^{89})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>57.17 ± 3.94 (^{91})</td>
<td></td>
</tr>
<tr>
<td>Central Corneal thickness (µm)</td>
<td>504 ± 40 (^{87})</td>
<td>503 ± 34.15 (^{88})</td>
<td>493.17 ± 42.84 (^{89})</td>
</tr>
</tbody>
</table>

**Table 1-1:** The common, diagnostic topographic and tomographic parameters used in the identification of keratoconus with respective, representative references.
In a study performed by Ambrosio et al.\textsuperscript{92} using the Pentacam, it was noted that the corneal-thickness spatial profile, corneal-volume distribution, percentage increase in thickness, and percentage increase in volume were different between keratoconic corneas and normal corneas and could serve as indices to diagnose keratoconus and screen refractive surgery candidates.\textsuperscript{92}

The introduction of wavefront analysis of the cornea has provided an additional tool for the diagnosis of keratoconus with widespread use of Zernike polynomials for characterization of wavefront aberrations of the human eye and description of the corneal surface. The measurement of higher order aberrations have been identified as a useful tool for identifying and grading keratoconic changes using the Orbscan II,\textsuperscript{93} Pentacam (Figure 1-10)\textsuperscript{94} and other videokeratoscopes.\textsuperscript{95, 96, 97}

Coma-like aberrations are known to be dominant in keratoconic eyes,\textsuperscript{95} and this parameter has been shown to have a high sensitivity when discriminating between normal and keratoconic eyes.\textsuperscript{93} The coma like aberration is due to the displacement of the optical axis by the keratoconic cone.\textsuperscript{96} In studies comparing aberrometry between normal and keratoconic eyes significant differences were noted in all anterior surface Zernike aberrometry parameters, except horizontal primary and secondary coma.\textsuperscript{94} Analysis of the posterior corneal surface, reveals statistically significant differences between groups in primary spherical aberration, primary vertical coma, coma RMS and coma-like RMS.\textsuperscript{94}

The increase in higher order aberrations (both corneal and total) in keratoconus has been noted to be a substantial 3.74 times higher on average than normal and that there is good correspondence between the anterior and total aberrations, indicating that the overall aberration pattern is dominated by the front surface of the cornea.\textsuperscript{95}
Figure 1-9: Scheimpflug image of a keratoconic cornea, measured with the Pentacam Rotating Scheimpflug tomographer (Oculus, Wetzlar, Germany).

Figure 1-10: The Zernike polynomial analysis map in a patient with keratoconus, measured with the Pentacam Rotating Scheimpflug tomographer (Oculus, Wetzlar, Germany).
1.7 TREATMENT OF KERATOCONUS

1.7.1 Spectacles and Contact Lenses

Until relatively recently the treatment methods for the visual disability of keratoconus were restricted to optimized optical correction (frequently contact lenses) until the disease stabilized or progressed to a degree severe enough to warrant surgical intervention.

In the early stages of keratoconus, vision is initially fully correctable with complex spectacle prescriptions, which, due to the irregular nature of the corneal surface, often provide increasingly poor visual function as the disease progresses. Spectacle intolerance due to high prescriptions can often be alleviated using either conventional or disposable soft toric contact lenses. When functional vision may no longer be achieved with these treatments, rigid gas permeable (RGP) lenses are required (Figure 1-11). Seventy four percent of keratoconic subjects in the CLEK study wore contact lenses in both eyes, highlighting the importance of this treatment modality.16

Figure 1-11: A. A micro-corneal Rigid Gas Permeable (RGP) contact lens in a keratoconic patient. B. A semi-scleral RGP contact lens in a keratoconic patient, examined using fluroscein dye to assess fit.
RGP lenses often have significant compliance and comfort issues unless fitted correctly. In the CLEK study 16.5% of subjects reported irritation due to RGP lens wear. RGP lens fitting still remains the mainstay of visual rehabilitation in these patients with the CLEK study reporting that 65% and 8% of the 1209 the subjects examined wore RGP lenses in both or one eye respectively. These authors also noted that 64% of the patients wore spectacles in some capacity at least some of the time.

In instances where the patient is intolerant to contact lenses or functional vision is no longer obtainable with refractive correction, surgical intervention is required to improve the vision.

### 1.7.2 Intra-Corneal Ring Segments (ICRS)

Intracorneal ring segments are small, semi-circular, inserts made of polymethylmethacrylate (PMMA) that are implanted in the corneal stroma with the aim of generating modifications in corneal curvature (Figure 1-12).

![Figure 1-12: A, B. Anterior segment imaging of Intra-corneal ring segments in a keratoconic patient. C. Optical section of the inferior ring of Intra-corneal ring segments in a keratoconic patient.](image)

Intra-corneal ring segments for the treatment of keratoconus, was first described by Colin et al in 2001. Originally a treatment for low myopia and myopic astigmatism in normal
corneas, the study reported this technique as an alternative to penetrating keratoplasty in contact lens intolerant patients with moderate keratoconus.\textsuperscript{98}

Clinical studies of ICRS (Addition Technology Inc., Des Plaines, Illinois, USA) in patients with keratoconus have reported improvements in spherical equivalent ranging from 2.23 to 3.69 D\textsuperscript{98-102}, improvement of cylinder dioptic power ranging from 1.30 to 2.54 D,\textsuperscript{98, 101} maximum keratometry improvement ranging from 2.14 to 4.60 D,\textsuperscript{98, 99} mean keratometric improvement ranging from 1.57 to 3.10 D,\textsuperscript{100, 101} and improvement in best spectacle-corrected visual acuity ranging from 1.4 to 2 lines.\textsuperscript{98, 100, 102}

These changes have been noted to be stable over time with studies reporting stability over 2 and 5 years of follow up.\textsuperscript{98, 100} However, in one study the central corneal thickness was reported to decrease from a mean of 478 µm to 421 µm two years following the procedure.\textsuperscript{103} This observation may be attributed to segment related corneal tissue stretching or simply to progression of the disease process which highlights the fact that ICRS do not stop or slow continued disease progression.\textsuperscript{104}

Recent studies have noted that patients with mild to moderate keratoconus (Kmax <53D) appear to be the best candidates for ICRS,\textsuperscript{99, 102, 105} with more advanced disease being more prone to complications such as intracorneal ring segment movement and dislocation.\textsuperscript{101} Contra-indications to INTACS implantation include central corneal opacities or a corneal thickness of less than 450 µm at the proposed insertion site.\textsuperscript{106}

Keraring technology (Ferrara Ophthalmics, Belo Horizonte, Minas Gerais, Brazil), is similar to ICRS in essence, though these inserts were designed specifically for keratoconus, whereas, ICRS evolved from myopia correction. Results for Keraring techniques are very promising in selected cases of early to moderate keratoconus.\textsuperscript{107-109}
1.7.3 Corneal Transplantation

Vision compromising corneal scarring in keratoconus may occur as a consequence of poor contact lens fitting, progression of the disease, or following corneal hydrops. Corneal transplantation is indicated in these cases and where adequate vision cannot be obtained with the best optical correction (Figure 1-13) (Figure 1-14).

Approximately 20% of keratoconic eyes will progress to penetrating keratoplasty, and in New Zealand keratoconus is the leading indication for penetrating keratoplasty, as reported by the New Zealand National Eye Bank, with 41% of all corneal transplants being performed for this reason.

![Figure 1-13: A. Anterior segment imaging of a penetrating keratoplasty (PKP) with interrupted sutures in a keratoconic patient. B. Penetrating keratoplasty following removal of sutures.](image)

Over the last 15 years the traditional, now century old, penetrating keratoplasty approach for keratoconus has increasingly been replaced by deep anterior lamellar keratoplasty (DALK) in cases where there is no significant corneal scarring or history of hydrops.
The DALK procedure, although technically more difficult than penetrating keratoplasty, has the major benefit of preservation of the host Descemet’s membrane and endothelium. Thus DALK avoids the risk of endothelial rejection and should result in greater longevity of allograft survival.\textsuperscript{113}

\textbf{Figure 1-14:} A. Lateral view of an extreme keratoconic cone prior to penetrating keratoplasty (PKP), B. Lateral view of same patient post-penetrating keratoplasty demonstrates a dramatic reduction in both corneal curvature and anterior chamber depth.
REFERENCES


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Chapter 2: Diagnostic Techniques
2.1 VISUAL ACUITY

Measuring visual acuity is the essential first step in any ocular examination. Keratoconus results in reduced visual acuity due to ametropic changes and distortion to the ocular surface. Each patient in this study, prior to every examination, was assessed using a projected Snellen visual acuity chart, for unaided visual acuity, pinhole visual acuity and best corrected visual acuity (with spectacles or soft contact lenses). Visual acuity was measured with standardised dim lighting conditions at a distance of 6 metres. Visual acuity was converted to LogMAR for statistical analysis.

2.2 SLIT-LAMP BIOMICROSCOPY

The slit-lamp biomicroscope is the most commonly used instrument in ophthalmic practice for observing the anterior segment of the eye. The slit lamp provides precise and modifiable illumination and magnification of the eye and surrounding structures. Direct illumination of the image allows for high magnification view of the anterior eye. The ability of the slit-lamp to provide focussed narrow slit beams, allows us to gain an optic section of transparent tissues such as the cornea, and delineated thickness, and the principal layers of the corneal tissue. This is particularly important in keratoconus as changes in corneal tissue profile and thickness can be visualised early in the disease. Retroillumination techniques are particularly useful for assessing corneal opacities and for changes visible in the corneal stroma and endothelium. Retroillumination of the cornea from the iris, produces higher contrast images of opaque alterations of the cornea.

Broad beam illumination allows a general view of the ocular surface to be viewed under diffuse light. A digital camera can be attached to a beam splitter or one of the eye pieces to capture the images (as demonstrated throughout this thesis). In these studies the slit lamp
used was a Topcon SL-7 (Itabashi-ku, Tokyo, Japan) (Figure 2.1), utilising image acquisition software and storage device Imagenet 2000, R2.60 Topcon (Itabashi-ku, Tokyo, Japan). Through variations in electronic flash and fill light (diffuse illumination accessory to the camera), the examiner was able to capture general information about the eye and document change in ocular structures between visits.

Vital dyes such as fluorescein are essential in any ocular examination and are used in conjunction with the slit lamp biomicroscope. Fluorescein allows visualisation of the tear film, and stains areas of missing epithelium made more visible with cobalt blue fluorescence. Fluorescein is also a good indicator in the assessment of an RGP contact lens fit. (Figure 1-11) Cobalt blue filters intrinsic to the slit lamp are also useful when examining deposits located in the cornea, such as in Fleisher’s ring in keratoconus, where the iron deposits in the deep epithelium completely or partially encircle the cone. (Figure 1-6)

Figure 2-1: Topcon SL-7 slit lamp (Itabashi-ku, Tokyo, Japan) with digital camera attachment
2.3 COMPUTERISED CORNEAL TOMOGRAPHY 
(ORBSCAN AND PENTACAM)

The Orbscan II tomographer (Bausch and Lomb, Rochester, NY, USA) uses a combination of Placido disc and slit beam scanning technologies (Figure 2-2). The Placido disc system analyses the image of the mires reflected from the cornea in terms of size, shape and position to extrapolate data regarding the corneal power and anterior corneal surface. The addition of the slit beam scanning system allows for the imaging of the anterior and posterior corneal surface, as well as the anterior lens surface and iris. The principle of triangulation\(^1\) allows the system to analyse the resulting data points to directly assess both elevation data from the two corneal surfaces and thereby corneal thickness. Further processing of the data allows for digital reconstruction of the anterior iris and lens. Notably, the triangulation process for the posterior corneal surface, anterior iris and lens is not as accurate as that of the anterior corneal surface due to hardware and operator limitations and the influence of the anterior surface measurement accuracy. \(^1\)

Acquisition of the scan begins with the subject’s chin being placed on the chin rest and forehead against the forehead bar. (Figure 2-2) The operator may secure the patient’s head further with a velcro strap. The subject is asked to maintain steady fixation on a fixation target within the instrument and to avoid blinking for the duration of the scan. The operator manually triggers the acquisition sequence after appropriate alignment of the instrument. During the acquisition, the Placido disk is illuminated and the mire’s reflection from the anterior corneal surface is stored (Figure 2-2). Subsequently, 40 slits, 20 from the right and 20 from the left, each 12.5 mm high and 0.3 mm wide, are projected onto the cornea at an angle of 45 degrees to the instrument axis. As the light from these slits passes through the cornea, it is scattered in all directions, but, crucially, it is backscattered toward the digital video camera of the device, which records the appearance. \(^2\)^\(^3\)
Data are typically represented in a quad-map format (Figure 2-3). Axial or tangential keratometric maps, anterior and posterior elevation maps, and wide field pachymetry maps are the four most common and practical maps displayed. The axial keratometric map provides information on power (radius of curvature) measurements across the cornea and can describe how the cornea performs in a (global) refractive sense.

Figure 2-2: The Orbscan II tomographer (Bausch and Lomb, Rochester, NY, USA) A. The illuminated Placido ring pattern B. The Placido image on the corneal surface. C, D. The Orbscan II in clinical use.

Tangential curvature, also referred to as instantaneous curvature, is calculated from single plane and multiple axes. The plane runs from a central point radially to the periphery, as in
the sagittal map, but the curvature is calculated by the tangent circle at each point along the plane.  

Tangential curvature has been presented as a better indicator of subtle (local) corneal topographic abnormalities.

Elevation provides information regarding the corneal surface in relation to the best fit sphere; a sphere that can be altered in terms of radius of curvature and position relative to the instrument axis to best fit the surface measured. The system generates a best fit sphere (mm) for both the anterior and posterior surface. Deviations of the measured surfaces from this sphere are represented as relative elevation or depression in microns (µm).

Figure 2-3: Standard quad-map in severe keratoconus measured with the Orbscan II tomographer (Bausch and Lomb, Rochester, NY, USA).

A study by Cairns and McGhee ¹ analysed the accuracy of Orbscan II measurements when compared to reference objects of known radius. They reported that despite statistically
significant differences between the Orbscan II measures and the Talysurf reference values, the magnitude of anterior elevation errors in was extremely small, less than 0.2 µm centrally and 0.7 µm peripherally. 

Early studies using Orbscan II noted that central corneal thickness was overestimated by 23 to 28 µm when compared to ultrasound pachymetry. This was thought, in part, to be due to the lack of tear film interface following the application of the probe in ultrasound pachymetry. Other factors such as probe misalignment and tissue compression during measurement with the ultrasound pachymeter are also potential sources of error.

The Pentacam Rotating Scheimpflug tomographer (Oculus, Wetzlar, Germany) (Figure 2-4) utilises a combination of a slit illumination system and a Scheimpflug camera using blue light emitting diodes (LEDs) (475 nm UV-free), which rotates to analyse the anterior segment.

The subject is asked to place their chin on the chin rest and forehead against the forehead bar (Figure 2-4). The subject is then asked to maintain steady fixation on the centre of the fixation target and to avoid blinking for the duration of the examination. Following correct alignment and focus by the operator, the Pentacam automatically starts the acquisition sequence. The instrument acquires 25 slit images (varying from 12 to 50 depending on the settings) of the anterior segment in approximately 2 seconds. A static camera is placed in the centre to detect the pupil’s contours and to compensate for fixation drift (this captures and corrects for eye movements).

The slit beam section illuminated by the machine is imaged using the rotating camera (orientated according to the Scheimpflug principle), allowing for radial images to be acquired along the illuminated plane from the anterior surface of the cornea right up to the
anterior surface of the crystalline lens. The sectional images are then combined to produce a three dimensional image of the anterior segment.

To estimate the corneal power, the Pentacam measures geometrical elevations, which are then converted to curvature values in the form of axial (sagittal) curvature or instantaneous (tangential) curvature. Measured parameters include corneal pachymetry, corneal keratometry, anterior and posterior corneal curvature, corneal astigmatism and Scheimpflug photography of the lens (Figure 2-5).

Analyses of corneal volume, corneal wavefront aberrations, densitometry and anterior chamber depth are also provided by the Pentacam (Figure 2-5). The corneal elevation profile is automatically converted into corneal wavefront data using Zernike polynomials with an expansion up to the 10th order. 8
Figure 2-4: A,B. The Pentacam Rotating Scheimpflug tomographer (Oculus, Wetzlar, Germany). C,D. The Pentacam in clinical use.

The Pentacam has been shown to have good repeatability. In recent studies the Pentacam system has been shown to produce reliable and repeatable measures of anterior axial curvature, simulated keratometric reading (SimK), and tangential and axial peripheral corneal curvatures at both anterior and posterior surfaces.

The intra-session reliability of anterior surface BFS at the 5.0 mm and 8.0mm zones (Cronbach $\bar{\Omega}0.999$ and the intra-class correlation coefficient (ICC) of $\bar{\Omega}.998$), and the posterior surface BFS at the 5.0 mm and 8.0 mm zones (Cronbach $\bar{\Omega} \bar{\Omega}0.997$; ICC $\bar{\Omega}0.990$) are reported to be high. There was no significant inter-session difference in anterior and posterior BFS measurements.
However, poor repeatability was observed for aberrometry measurements where intra-observer repeatability and inter-observer repeatability were acceptable only for primary and secondary spherical aberration.  

This poor reliability in terms of calculating wavefront aberrations was also re-iterated by Shankar et al who noted that the Pentacam was not reliable in measuring corneal wavefront aberrations, largely due to variability in corneal elevation data.
Figure 2-5: A,B. Standard clinical maps of keratoconus measured with the Pentacam Rotating Scheimpflug tomographer (Oculus, Wetzlar, Germany).
2.4 CORNEAL PACHYMETRY

Corneal pachymetry can be measured using computer-assisted corneal topography/tomography, such as the Orbscan II tomographer (Figure 2-6) or the Pentacam rotating Scheimpflug (Figure 2-7) corneal tomographer which produce wide field pachymetry maps as noted in the preceding section.

The Orbscan II pachymetry map analyses (Figure 2-6) the elevation difference between anterior and posterior corneal surfaces and presents summary data within 5 or 9 circular areas of 2.0mm diameter each. These circles are located in the centre of the cornea and at 4 or 8 locations in the mid-periphery (superior, supero-temporal, temporal, infero-temporal, inferior, infero-nasal, nasal, and supero-nasal), each 3.0mm from the central instrument axis. The Orbscan software also recognizes the thinnest point of the cornea and marks its distance from the instrument axis and its quadrant location. 16

![Corneal pachymetry map measured with the Orbscan II tomographer (Bausch and Lomb, Rochester, NY, USA).](image)

**Figure 2-6:** Corneal pachymetry map measured with the Orbscan II tomographer (Bausch and Lomb, Rochester, NY, USA).
Theoretically, the Orbscan II system provides more accurate corneal thickness readings by applying an acoustic equivalent factor, related to the readings obtained by ultrasound. However, the acoustic factor of 0.92 is a mean value applied to all Orbscan thickness measurements and although this allows for correlation between the measurements, it is less likely to be accurate in very thin or very thick corneas. Orbscan II has been shown to correlate well with the Pentacam rotating Scheimpflug tomographer when the acoustic factor is used. 

Other investigations of Orbscan II accuracy compared to ultrasound pachymetry (before the correction factor was applied) highlighted that the Orbscan overestimated the corneal thickness across the cornea, with the mean difference 48.15 µm ± 33.74. The study noted that the differences increased toward the periphery, and that the reliability of Orbscan readings decreases with increased corneal thickness. Of relevance to the current study, corneal thickness measurements in keratoconus have shown that Orbscan II measurements are significantly thinner than those of ultrasonic pachymetry. However, because it is based on optical analysis, Orbscan pachymetry measurements are likely to be affected by a loss of transparency in the cornea (e.g., haze). In corneas with clinically significant haze, corneal thickness measurements tend to be lower when measured using Orbscan II compared to ultrasound pachymetry. In a study investigating the effects of post refractive surgery haze (post- LASIK and post- PRK), it was noted that the Orbscan II significantly underestimated the corneal thickness when compared to ultrasound pachymetry. The authors also noted a significant correlation between the grade of haze after PRK and the decrease in Orbscan pachymetry measurements and the increase in posterior surface elevation in the elevation maps of the Orbscan II measurements. This difference has been attributed to the acquisition
technique used by the Orbscan, with any haze resulting in a high level of light scatter with an ensuing increase in the refractive index of the cornea.  

![Figure 2-7: Corneal pachymetry map in a keratoconic patient measured with the Pentacam Rotating Scheimpflug tomographer (Oculus, Wetzlar, Germany).](image)

The ultrasound (US) Pachymeter is often referred to as the gold standard instrument for measuring corneal thickness. However, traditional US pachymeter is far from perfect as it measures a very small area of the corneal surface and multiple measurements are required across the cornea in order to obtain an impression of the global thickness of the cornea.  

Other sources of variability include misalignment of the probe and its direct bearing on the cornea potentially causing tissue compression. US pachymetry also has the disadvantages of the need for topical anaesthesia and contact of a probe with the cornea, however, US pachymetry is reported to have small intra-examiner variability.  

A study by Grewal et al analysing the central corneal thickness in keratoconic and normal eyes using the Pentacam, ultrasound pachymetry and anterior segment optical coherence tomography (AS-OCT) noted significantly higher measurements with the US pachymeter
(mean 446.4 ± 57.9 mm) than with the Pentacam (mean 439.6 ± 62.1 mm) and AS-OCT (mean 441.8 ± 58.4).\textsuperscript{23} However, Amano et al\textsuperscript{9} showed that the Pentacam correlates well with US pachymetry and the Orbscan II pachymetry (with the acoustic factor) in normal eyes.\textsuperscript{9} The repeatability and reproducibility of Pentacam measures of anterior power, posterior power, and total corneal power in normal eyes have been shown to be higher than that of the Orbscan II.\textsuperscript{24} Data have recently been published on the Pentacam highlighting no significant differences in corneal values across several different international, ethnically diverse, populations.\textsuperscript{25}

### 2.5 IN VIVO CONFOCAL MICROSCOPY

The ability to examine the microstructural alterations in keratoconus was previously limited to ex-vivo analysis of diseased corneal buttons following corneal transplantation. However, with the introduction of in vivo confocal microscopy (IVCM) has enabled examination of the living human cornea at the cellular level.

The Heidelberg Retina Tomograph II Rostock Corneal Module (Heidelberg Engineering, GmbH, Germany) (Figure 2-8) is a laser scanning in vivo confocal microscope that uses a 670nm, coherent light Helium Neon diode laser source. Two scanning mirrors deflect the laser beam in two perpendicular directions scanned sequentially over each point in the examined area.\textsuperscript{26} The mirrors scan the beams horizontally and vertically to produce respective scan fields. De-scanning of reflected light is performed by the same two scanning mirrors.\textsuperscript{26} The reflected light is deflected to a photo diode and subsequently collected and digitized to form the image.\textsuperscript{26}
When a 60x objective water immersion lens with a numerical aperture of 0.9 (Olympus, Tokyo, Japan) is used, the IVCM images that are produced are 400µm x 400µm in dimension, with a lateral resolution of 2µm and optical section thickness of 4µm.

**Figure 2-8**: A,B. The Heidelberg Retina Tomograph II Rostock Corneal Module (Heidelberg Engineering, GmBH, Germany) C. The Rostock corneal module in clinical use.

In practice both eyes are anesthetised using 0.4% benoxinate hydrochloride (Chauvin Pharmaceuticals, Surrey, UK) topical anaesthetic prior to examination. A coupling agent, Carbomer 980 (Viscotears 0.2%; Novartis, North Ryde, NSW, Australia) is placed between the lens and the applanating lens cap and patients are asked to fixate at a standard distance target, and the lens is manually advanced toward the cornea until applanation is achieved without compression (Figure 2-8). The full thickness of the cornea may be examined using the section mode, where single images are manually acquired by the
observer at any desired corneal depth (Figure 2-9). The quality of the IVCM images have been reported to be reliable for the calculation of epithelial, keratocyte, and endothelial cell densities with inter-examiner 95% limits of agreement of ± 7.9%. 27
Figure 2-9: The Heidelberg Retina Tomograph II Rostock Corneal Module (Heidelberg Engineering, GmbH, Germany): A. Epithelium: Consists of five layers of cells measuring 50-60µm thick

B. Sub-basal nerve plexus, with fine branching patterns visible in a normal patient.

C. Anterior stroma, with densely packed keratocyte nuclei and a visible stromal nerve.

D. Posterior stroma, showing the reduced density of keratocyte nuclei present in the posterior stroma.

E. Endothelium, Showing the normal hexagonal structure of the endothelium in a healthy, normal, cornea.
The Confoscan 4 (Nidek Technologies, Gamagori, Japan) (Figure 2-10) is a slit scanning confocal microscope. Two optically conjugate white light beams are used for illumination combined with a rapidly oscillating two sided mirror. The mirror scans the image from the slit beam onto the microscope and de-scans the reflected light from the objective.

Both eyes are anesthetized by instillation of topical 0.4% benoxinate hydrochloride (Chauvin Pharmaceuticals, Surrey, UK). The subject is asked to place their chin on the chin rest and head against the forehead bar (Figure 2-10). The subject is then instructed to fixate on an internal fixation target to enable examination of the central cornea. The tip of the objective lens is coated with a viscous contact solution Carbomer 980 (Viscotears 0.2%; Novartis, North Ryde, NSW, Australia), and the objective 40X gel immersion lens is advanced manually until the gel is in contact with the surface of the cornea. The operator is required to adjust the position of the lens until the field of view of the microscope is centred on the corneal stroma. Automated alignment and scanning is then initiated.

Figure 2-10: A. The Confoscan 4 (Nidek Technologies, Gamagori, Japan) in clinical use. B. An image of the corneal endothelium in a normal patient taken with the Confoscan 4.

Automatic scanning allows for multiple single section images to be acquired across the full thickness of the central cornea (Figure 2-11). Full-thickness confocal scans are acquired at
a speed of 25 frames per second, obtaining 350 images per scan with each image acquired at 5 μm depth intervals. The images are then transferred and stored on the NAVIS application (Nidek Technologies, Gamagori, Japan), where images can be viewed and analysed. Automated, semi-automated and manual techniques may be used to assess endothelial densities. Good intra-observer and inter-observer reproducibility of endothelial density measurement using automated endothelial analysis has been reported.²⁸
**Figure 2-11:** The Confoscan 4 (Nidek Tecnologies, Gamagori, Japan) slit scanning confocal microscopic imaging of A. Epithelium, A. Epithelium, showing basal honeycomb appearance

B. Sub-basal nerve plexus with vertical orientation of fibres in the centre of the image.

C. Anterior Stroma with highly reflective keratocytes

D. Posterior Stroma highlights a lesser density of keratocytes and a vertically orientated stromal nerve

E. Endothelium with healthy, densely packed, hexagonal cells.
2.5.1 IVCM of Corneal Epithelium

Uçakhan et al. noted elongated, desquamating superficial epithelial cells, a feature that was more pronounced with increasing severity of keratoconus. In the moderate stages of keratoconus, superficial epithelial cells appear normal; however in cases of severe keratoconus irregular superficial epithelial cells with an elongated or spindle-like shape have been observed. Spindle-shaped epithelial cells are characteristic of the wound-healing response and associated cellular migration.

Brightly reflective material deposited within the basal epithelial cells has also been reported, which is thought to represent the haemosiderin deposition associated with a Fleisher’s ring. Hollingsworth et al. noted that the mean diameter of wing cell nuclei and basal cells in keratoconic patients was significantly greater than that of the normal population.

In patients with severe keratoconus, the wing cell layer was shown to have displaced, large, and irregularly spaced nuclei and the cells were significantly larger in diameter than that of normal corneal wing cells. Normal basal epithelial cells were seen in mild to moderate keratoconus, but in more advanced disease the basal cell layer was hazy and irregular in appearance, with large cells and faint cell borders. Basal epithelial cell density is significantly decreased in keratoconus compared to non-diseased corneas. Niederer et al. noted a correlation between severity of disease and basal epithelial cell densities.

2.5.2 IVCM of Bowman’s Layer

Varying degrees of irregularity are observed in Bowman’s layer. In some corneas, with no clinical signs of scarring, Bowman’s layer appears normal. Whereas in other more severely diseased corneas, Bowman’s layer in the area of the corneal apex may actually appear to
be absent. ³⁰, ³¹ Haze or hyper-reflective patches at the level of Bowman’s layer may be observed on in vivo confocal microscopy and are representative of scarring. ³⁰

2.5.3 IVCM of Sub-Basal Nerve Plexus and Corneal Nerves

The central sub-basal nerve plexus density is significantly reduced in keratoconic patients when compared to normal, ²⁷, ³⁴, ³⁶, ³⁷ and correlated with disease severity. ²⁷ Mocan et al. noted thicker sub-basal and stromal nerves in the keratoconic corneas analysed. ³⁴ Ucakhan et al noted that thickened and prominent corneal nerves were visible in 29.2% of the keratoconic corneas they examined, and were more evident with increasing disease severity. They also noted that 31.3% of the keratoconic eyes they examined had excessive branching and curling of the sub-basal nerve plexus (Figure 2-12), however, the images in this study were only obtained from the central cornea. ²⁹

Figure 2-12: Sub-basal nerve plexus imaged with the Heidelberg Retina Tomograph II Rostock Corneal Module (Heidelberg Engineering, GmBH, Germany) in A. A normal patient, and B. A keratoconic patient.
Patel and McGhee described a technique for producing wide field montages of the sub-basal nerve plexus using multiple overlapping *in vivo* confocal microscopy images (Figure 2-13). Using this technique in patients with keratoconus and no history of contact lens wear, a distinctly abnormal configuration of the sub-basal nerve plexus was observed when compared to the normal sub-basal nerve architecture.

**Figure 2-13:** Schematic showing a wide-field montage of the sub-basal nerve plexus in a case of moderate keratoconus
Instead of a radiating pattern, a tortuous network of nerves was noted in keratoconus. When the montages were superimposed to scale onto the patients corneal topographic maps, the nerve fibre bundles appeared to follow the contours of the base of the cone.  

2.5.4 IVCM of Corneal Stroma

The presence of corneal scarring on slit lamp examination is significantly related to the degree of haze seen on in vivo confocal microscopy in both the anterior and posterior stroma, and in some cases has been shown to correlate to areas of apical touch with rigid contact lens wear. Regression analysis was employed by Hollingsworth et al to identify the associations of haze (quantified using the author’s own grading scale) on the apparent keratocyte density in the anterior and posterior stroma. They noted that increasing levels of haze were associated with a reduction in the apparent keratocyte density, with a larger correlation noted in the anterior than the posterior stroma. However, the degree of stromal haze did not correlate with disease severity as classified by corneal curvature. Hyper-reflective and irregularly shaped stromal keratocytes are also a commonly observed feature in keratoconus.

The mean anterior and posterior keratocyte density is reduced in keratoconic corneas when compared to normal. Anterior keratocyte density is positively correlated with central corneal thickness and inversely correlated with keratometry. Interestingly, anterior and posterior keratocyte density is lower in rigid gas permeable contact lens wearers (both normal and keratoconic).

Anterior and posterior stromal keratocyte density has been reported to be between 12-39% and 10-27.5% lower respectively when compared to normal populations.
The presence of corneal staining, atopy and a history of eye rubbing have been significantly associated with lower anterior keratocyte density, but not posterior keratocyte density.\textsuperscript{33} The presence of alternating dark and light bands in the stroma appear to correspond to the appearance of Vogt's striae on slit-lamp biomicroscopy examination (Figure 2-14). \textsuperscript{30}

![Figure 2-14](image)

**Figure 2-14:** Clinical imaging of Vogt's striae at the apex of a keratoconic cornea, with A. Slit lamp photography, B. The Confoscan 4 (Nidek Tecnologies, Gamagori, Japan) slit scanning confocal microscope.

2.5.5 IVCM of Descemet's Membrane

There are no abnormalities at the level of Descemet's membrane in keratoconus when observed using \textit{in vivo} confocal microscopy. \textsuperscript{30}

2.5.6 IVCM of Endothelium

There is conflicting evidence regarding corneal endothelial density in keratoconus. Hollingsworth et al. reported significantly increased endothelial cell density (6%) in keratoconic corneas when compared to normal. \textsuperscript{33} Other groups have shown reduced
endothelial cell density in keratoconus, 
whilst other studies have identified no significant differences in the endothelium (Figure 2-15).

Figure 2-15: The Confoscan 4 (Nidek Tecnologies, Gamagori, Japan) slit scanning confocal microscopic imaging of the corneal endothelium in: A. A normal patient, B. A Keratoconic patient C. A patient with Fuchs endothelial dystrophy.

2.6 OCULAR RESPONSE ANALYSER

2.6.1 Principles of Ocular Response Analysis

The Ocular Response Analyser (ORA; Reichert Technologies Inc., Depew, New York, USA) measures intraocular pressure (IOP) and biomechanical properties of the cornea in vivo (Figure 2-16). The ORA utilises an air-puff method similar to that of the traditional non-contact tonometer. The pressure from the air puff causes the cornea to indent, moving past a first applanation event, into a slightly concave shape. The air puff is then switched off and the pressure applied to the eye is decreased in an inverse-time, symmetrical fashion. As the pressure decreases on the cornea, it passes through a second applanation state and then returns to the normal corneal curvature.

As one single measurement on the cornea cannot take into account both the corneal biomechanical properties and the force of the IOP, two measurements take place. These
occur over a 20 millisecond time course so as to negate the possible influences of eye position and movement and ocular pulse amplitude.  

Figure 2-16: The ocular response analyser in clinical use.

An electro-optical collimation detector system monitors the corneal curvature in the central 3.0 mm diameter throughout the 20 millisecond measurement. A filtered (smoothed) version of the detector signal defines two precise applanation times corresponding to two well-defined peaks produced by inward and outward applanation events. Two corresponding pressures of an internal air supply plenum are determined from the applanation times derived from the detected applanation peaks. These two pressures are defined as the intersection of a vertical line drawn through the peaks of the applanation curve with the plenum pressure curve.

The values of the plenum pressure signal at the two applanation times (applanation pressures) are different; thought to be primarily because of the viscoelastic properties of the cornea causing a delay in the two applanation events. The difference between the two pressures is termed the Corneal Hysteresis (CH); a measure of inherent viscous damping in the cornea (P1· P2) (Figure 2-17). CH is used to calculate two other measures; Corneal Resistance Factory (CRF) and Corneal-compensated IOP (IOPcc). CRF predominantly
relates to the elastic resistance properties of the cornea, giving a measure of its overall rigidity or resistance and is derived from the formula \((P1 - kP2)\), where \(k\) is a constant.\textsuperscript{41, 42} The constant \(k\) was determined from an empirical analysis of the relationship between both \(P1\) and \(P2\) and CCT in order to develop a corneal parameter more strongly associated with CCT than CH. (personal communication, D. Luce, Reichert Corporation, August 2010).

\(IOP_{CC}\) is another useful measure as it is less affected by corneal properties and thus is thought to provide a better indicator of the true IOP than the traditional Goldmann tonometer measurements. The ORA also provides a Goldmann-correlated IOP value (\(IOP_{G}\)) by averaging the inward and outward applanation pressures.

Ortiz et al.\textsuperscript{43} showed that in normal eyes, CH and CRF decreased with age; however, the differences were only statistically significant for CH between the youngest group (<14 years) and the oldest group (>60 years). The authors suggested that this relationship implies a change in the elastic properties of the cornea with increasing age. However, No linear correlation was observed between age and CRF.\textsuperscript{43}
Corneal biomechanical strength is known to be reduced in keratoconic corneas. The ability to measure the corneal strength \textit{in vivo} has previously been limited to extrapolations from corneal thickness measurements. However, the introduction of the Ocular Response Analyser (ORA) has led to a dramatic increase in research investigating the effects of corneal disease and surgical procedures on the biomechanical properties of the cornea.

In the normal population, mean CH values of 9.9 to 11.0 mmHg have been reported\textsuperscript{43, 45, 46} In keratoconus, typically lower mean CH values of 7.7 to 9.6 mmHg have been reported (Figure 2-18).\textsuperscript{43, 45, 46, 47, 48, 49, 50, 51} The mean CRF value ranges from 10.1 to 11.1 mmHg in normal populations,\textsuperscript{43, 47, 49, 48, 52} compared to lower mean values ranging from 6.2 to 7.5 mmHg in keratoconic populations (Figure 2-18).\textsuperscript{43, 47, 49, 48, 52}
Figure 2-18: An Ocular Response Analyser signal in A. A normal patient. B. A keratoconic patient with a reduced signal profile and peak pressures.

Corneal Hysteresis and Corneal Resistance Factor have been reported to be significantly reduced in keratoconus. 40, 43, 45, 48, 49, 51, 52 However, because of the large overlap between normal and keratoconic corneas, CH and CRF have a low sensitivity for discriminating between the early stages of keratoconus and normal corneas. 43, 45, 47, 51, 52, 147 Although study of ORA measurements in keratoconus have revealed significant differences in the corneal hysteresis between normal corneas and moderate to severe keratoconus, no significant differences were noted between normal and mild keratoconus, mild and moderate, or moderate and severe keratoconus. 41

Alio et al 53 noted a significant negative correlation between a) CH and keratoconus grade based corneal higher order aberrations (Alió and Shabayek modification of the Amsler-Krumeich keratoconus scale) 53 and b) between CRF and the keratoconus grade i.e. the higher the keratoconus grade, the lower the CH and CRF values. The majority of
participants in this study had grade 1 or mild keratoconus; however the authors did not take into account the variations in central corneal thickness measurements between the keratoconic groups. 54

In contrast to the majority of studies, a recent study has noted that there is a significant difference in the mean CH and CRF between normal and Forme Fruste Keratoconus (FFKCN) corneas after controlling for differences in age, gender, and central corneal thickness, which are known factors to influence keratoconus. 49

In a recent in vivo confocal microscopy study, keratocyte density in the anterior half of the stroma, posterior half of the stroma, and full thickness stroma were all negatively correlated with CH and CRF in healthy eyes. However, interestingly there was no significant relationship between either CH, CRF, and keratocyte density in eyes with keratoconus. 50
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Chapter 3: Corneal Collagen Cross-Linking
3.1 CONTACT CROSS-LINKING

The mechanical strength and rigidity of the cornea has been shown to be reduced in keratoconus due to reduced cross-links and corneal tissue thinning. This decreased rigidity has been thought to result in the increased distensibility and subsequent protrusion of the cornea in keratoconus. The reduced number of Cross-links between collagen fibrils in keratoconus has been confirmed using immuno-histochemistry techniques.

Recently, corneal collagen cross-linking has been introduced as a new method of treating keratoconus. This method involves inducing cross-links between collagen fibrils in the cornea, aiming to increase the mechanical rigidity, thereby allowing the tissue to resist further protrusion. This treatment method is only indicated in early to moderate keratoconus, aiming to halt or slow down the progression of disease at a stage when the vision can still be maximized with spectacles or contact lenses.

3.2 MECHANISM OF ACTION

Cross-linking refers to the formation of links between polymer chains. Photo Cross-linking generates highly reactive intermediates by irradiation of an inert precursor using ultra-violet light. In the normal cornea, cross-links (covalently bonded molecular bridges) act to bind adjacent tropocollagen helices, micro fibrils and fibrils. Naturally occurring corneal cross-linking has been shown to increase with age and in some disease processes such as diabetes due to glycation induced cross-linking of collagen molecules. The mechanism of action is thought to be the advanced Maillard reaction which begins with the addition of aldehyde sugar to amino groups on proteins. This combination forms a condensation product which is subject to rearrangement and reactions to form advanced glycosalation end products (AGEs).
Natural cross-links occur in diabetic patients due to non-enzymatic glycosylation triggered by interstitial glucose.\textsuperscript{15, 16} Patients with diabetes have been noted to have a lower risk of developing keratoconus.\textsuperscript{14} Whilst some studies have reported that diabetes is not associated with a diagnosis of keratoconus, it does at least appear to decrease the odds of having more severe keratoconus.\textsuperscript{17}

Cross-linking is a widely used method in many different industries to harden or strengthen materials. For example, in the polymer industry cross-linked polyethylene is utilized in water piping and insulation of high tension electrical cables. Cross-linking of these materials improves mechanical strength and chemical resistance.\textsuperscript{18} Cross-linking techniques have also been used for the preparation of prosthetic heart valves and in dentistry to harden filling materials.\textsuperscript{18, 19}

Early investigations into the induction of cross-links in the cornea revealed that ultraviolet (UV) light alone does not stimulate cross-links in the porcine cornea\textsuperscript{18} and the authors attributed this to the poor penetration of the UV light through the cornea. A photosensitizer was therefore required; in this circumstance riboflavin was selected as it is soluble in water and penetrates into the cornea. Previous studies had reported that the active species of oxygen generated by the combination of riboflavin and UV light stimulated cross-linking in bovine vitreous collagen \textit{in vitro}.\textsuperscript{20}

Corneal collagen cross-linking uses riboflavin which is excited by UVA light at a wavelength of 365nm and subsequently excited into its triplet state (Figure 3-1). This multiplicity promotes the generation of the reactive oxygen species (ROS), singlet oxygen and to a lesser extent superoxide anion radicals (Figure 3-1).\textsuperscript{19} Cross-links are then stimulated between the amino groups on the collagen fibrils in the anterior corneal stroma.\textsuperscript{19}
The wavelength of UVA light was chosen to match riboflavin’s peak absorption at 365nm. Experiments investigating the effects of riboflavin and sunlight exposure on porcine corneas showed a significant increase in mechanical stiffening as measured by the stress-strain measurement. Spoerl et al subsequently suggested that a modification of this technique may be used to stiffen the human cornea.

![Diagram](image)

**Figure 3-1:** The principle of photodynamic crosslinking by ultraviolet A and the photosensitizer riboflavin (after Wollensak)

Early ex-vivo studies have investigated the cross-linking efficacy of other chemical agents and approaches such as Karnovsky’s solution, gluteraldehyde and riboflavin, in combination with different light sources including UV-light (365 nm), blue light (436 nm) and natural sunlight in porcine cornea. Spoerl et al noted that when compared to untreated corneas, treatment with gluteraldehyde, Karnovsky’s solution and combined riboflavin and UV-irradiation all resulted in significantly increased mechanical stiffness as measured by stress-
strain measurements. These investigators therefore established that the mechanical stiffness of the cornea can be artificially increased using either aldehydes or a photosensitizer activated by ultra-violet light, and that photosensitizers are required to induce cross-linking as UV alone is poorly effective.

Riboflavin has a molecular weight of 376.37 g/mol and is water soluble; therefore a hydrophobic (lipophilic) structure such as the corneal epithelium limits the diffusion of this drug. Vitamin B2 is usually well tolerated and is a non-toxic water soluble photosensitizer that has been shown to penetrate easily into the stroma in absence if the epithelium.

3.3 EFFECTS ON EX-VIVO TISSUE

Spoerl et al investigated the mechanical stabilization effects of riboflavin and UVA and gluteraldehyde on ex-vivo rabbit corneas. The results showed an increase in mechanical stability by a factor of 1.3 ± 0.66 measured by stress strain measurements in the gluteraldehyde group and a factor of 1.6 ± 0.75 in the riboflavin group at 1 month following treatment. The collagen Cross-linking effect described in these studies has been reported to have a similar effect to that of formaldehyde when used to fix and preserve biological specimens.

A study investigating the stress-strain in human and porcine corneas after undergoing the UVA and riboflavin collagen Cross-linking procedure noted an increase in biomechanical rigidity, as indicated by Young’s modulus, by 71.9% in porcine eyes and a substantial 328.9% in human corneas.

The different degree of increased rigidity in the two tissue types is related to the difference in thicknesses between the porcine and human tissue. The anterior portion of the cornea
treated amounts to 35% cross-linking volume in the porcine cornea (300 µm/850 µm) and 54% cross-linking volume in the human cornea (300 µm/ 550 µm), therefore, a greater proportion of the human cornea is affected by the procedure.  

In a study investigating the corneal collagen composition after the collagen cross-linking process using gel electrophoresis, a polymer band of Cross-linked type I collagen of at least 1000-kDa molecular weight was identified which was thought to represent the increased collagen fibre diameter and therefore confirmed the efficacy of the process. This polymer band demonstrated significant mechanical resistance to denaturation by heat, pepsin, and mercaptoethanol treatment. Similar observations were also made in a study which highlighted that corneas treated with combination riboflavin and UVA light had a two times increase in digestion time when digested with enzymes such as pepsin, trypsin and collagenase.  

3.3.1 Distribution of Corneal Collagen Cross-Linking

Studies have outlined the distribution of the changes in rigidity in the cornea following cross-linking, and noted that the majority of the effect is isolated to the anterior 300-350 µm of the corneal stroma. Studies using human and porcine donor tissue have shown that the cornea is only significantly stiffened in the anterior 200 µm of the stroma, and that the stress of the anterior stromal tissue as measured by Young’s modulus was 70% higher than in the posterior stromal tissue in untreated corneas. This is thought to indicate that the majority of the increased corneal rigidity comes from the anterior stroma.

Anterior corneal collagen fibre diameter in rabbit corneas, following the cross-linking procedure, increased in diameter by a mean of 12.2% compared with the untreated fellow control eyes. In the posterior stroma, the collagen fibre diameter increased by 4.6% when compared with the untreated fellow eye. Additionally, collagen fibres in the anterior stroma
were 9.3% thicker than those in the posterior stroma of the same eye.\textsuperscript{30} This increase in corneal collagen fibre diameter by a mean of 3.96nm, has been shown to be below the threshold level (150nm) required to cause loss in transparency in the anterior stroma of the cornea.\textsuperscript{31}

Further evidence for the anterior distribution of the collagen cross-links following corneal cross-linking has been outlined in studies investigating the hydration behavior of the cornea.\textsuperscript{32} In the \textit{un-hydrated} state, the anterior, densely cross-linked zone comprises the anterior 242µm of the cornea, the intermediate partially Cross-linked zone 238µm, and the non cross-linked posterior zone forms the posterior 135µm.\textsuperscript{32} Following hydration, the densely Cross-linked anterior zone was shown to have no swelling, whereas, the partially cross-linked intermediate zone exhibited a hydration factor of 2.2, and the posterior non cross-linked zone had a hydration factor of 2.7.\textsuperscript{32} The degree of swelling is directly influenced by the swelling of the collagen.\textsuperscript{32} Hydration behavior of the cornea is therefore a potential indicator for assessing the area and localization of collagen Cross-linking.\textsuperscript{32}

The preferential cross-linking of the anterior stroma may be attributed to the rapid dissipation of the UVA irradiance across the cornea due to an increased absorption by the photosensitizer riboflavin.\textsuperscript{30}

Measurements of the absorption coefficient of rabbit, porcine, and human corneas saturated with a 0.1% riboflavin concentration noted that riboflavin increased the absorption coefficient of ultraviolet light by a factor of 5, which limits the UV irradiance through a 400µm-thick stroma to 0.32 J/cm\textsuperscript{2} at the endothelial level which is well below the damage threshold of 0.65 J/cm\textsuperscript{2}.\textsuperscript{21}
3.4 *EX-VIVO* MEASUREMENTS OF THE MICRO-STRUCTURAL EFFECTS OF CORNEAL CROSS-LINKING

3.4.1 Light Microscopy

Light microscopy studies investigating cross-linking on post mortem eye bank corneas revealed superficial, highly reflective, spherical structures that were approximately 4–10 µm in diameter within the anterior 20 µm of the corneal stroma. These hyper-reflective structures were observed in the stroma up to a depth of 300 µm, although at lower densities than in the anterior stroma. The posterior corneal stroma (over 300 µm depth), and endothelium both appeared normal. The composition of these structures is unknown. 33

3.4.2 Immunohistochemistry

Immunohistochemical analysis was performed in keratoconic human corneal tissue treated with collagen-cross-linking 6 months prior to penetrating keratoplasty. A significant increase in collagen fibre diameter was subsequently identified in the anterior stroma of cross-linked corneas compared with healthy controls (22.6%) and keratoconus cases (16.1%). However, collagen fibre thickness was not significantly increased in the posterior stroma following cross-linking in comparison with normal and keratoconic corneas. 34

Laboratory studies on rabbit and human corneas reveal extensive loss of keratocytes at 24 hours post cross-linking treatment. An almost acellular anterior zone (with some remaining apoptotic, TUNEL positive or completely necrotic cells at variable depths of the corneal stroma) correlates with an increasing surface UVA irradiance ranging from 0.4 to 1.0mW/cm². An abrupt cytotoxic irradiance level has been identified at 0.5mW/cm² for
stromal keratocytes.\textsuperscript{28, 34} Esquenazi et al have demonstrated that the cytotoxic effect of cross-linking appears isolated to the anterior 250µm of corneal stroma.\textsuperscript{35}

Cross-linked corneas demonstrate keratocyte apoptotic changes, such as the formation of apoptotic bodies, chromatin condensation, and cell shrinkage.\textsuperscript{33, 28} Studies have also observed TUNEL-positive cells in the cross-linked area at days 1 and 3 after treatment. By day 10, no TUNEL positive cells were observed, indicating that the initial apoptotic event occurs in the first 7-10 days. All of these cellular changes appear only in the treated area with a sharp transition zone toward the normal-appearing adjacent untreated tissue.\textsuperscript{33, 28}

As previously noted, a recent study performed immunohistochemistry on corneal buttons removed from patients undergoing penetrating keratoplasty for keratoconus, who had previously undergone cross-linking 6 month earlier. Keratocyte proliferation was evaluated in these buttons using Ki-67 staining. Almost no Ki-67-positive cells were detected in the healthy corneas and positive cells were rare in keratoconus. However, in keratoconic corneas treated with cross-linking, there was a significant increase in the number of Ki-67-positive cells, and CD-34 positive cells (a marker for keratocyte phenotype) were regularly distributed throughout the whole corneal stroma showing a distribution similar to that observed in healthy corneas. This study provided the first evidence that in the human cornea, combined riboflavin/UVA cross-linking treatment stimulates both keratocyte apoptosis and promotes keratocyte repopulation. Upon injury to the cornea, keratocytes can transform into divergent phenotypes, which are dependent on specific environmental signals. This study also showed that after cross-linking the keratocytes were positively stained for CD34, but negatively stained for a-smooth muscle actin and for desmin (markers for myofibroblasts). Therefore the authors suggested that cross-linking treatment is not sufficient to induce keratocyte differentiation to myofibroblasts.\textsuperscript{34}
Keratocyte apoptosis is assumed to initiate a corneal wound healing response and start the complex cytokine cascade. Myeloperoxidase (MPO) positive cells or macrophages have been observed in the area adjacent to the treatment zone and in the transition zone of the corneal stroma, followed by migration of activated keratocytes approximately 10 weeks after treatment. \(^{35}\) In some instances increased density of the extracellular stromal matrix has been noted beneath the cross-linked treatment area along with persistence of myofibroblasts. Repopulating keratocytes reabsorb abnormal collagen and other matrix abnormal materials deposited by myofibroblasts allowing the cornea to restore its clarity. \(^{35}\)

The cytotoxicity level for keratocytes has also been calculated for the various stromal depths according to the Lambert-Beer law and was determined to be in the range of 0.49ι 0.77 mW/cm\(^2\) irradiance corresponding to UVA doses of 0.86ι 1.39 J/cm\(^2\). \(^{28}\) Immunohistochemistry studies have revealed a fluorescence line in treated corneas stained with type I collagen antibody located in the anterior 182.5 ± 22.5μm of post-mortem porcine cornea. This zone was further separated into a superficial zone with highly organized collagen fibres of 105 ± 15 μm and a posterior zone with partially organized fibres of 75 ± 10 μm. This was not identified in corneas where the epithelium had not been removed prior to treatment.\(^{36}\)
REFERENCES


SECTION II

Corneal Tomography in Keratoconus
Chapter 4: Computerised Corneal Tomography and Associated Features in a Large New Zealand Keratoconic Population
4.1 INTRODUCTION

Keratoconus is a non-inflammatory, progressive ectasia of the cornea that has a variably reported prevalence of between 50 and 230 per 100,000.\(^1\)\(^-\)\(^3\) An increased prevalence of this disease has long been postulated in the New Zealand population, evidenced by the high national rate of corneal transplantation for keratoconus compared with international data.\(^4\)

Reported associations with keratoconus include, family history, atopy and eye rubbing\(^5\) and in New Zealand the national prevalence of keratoconus may be influenced by high rates of eczema (15%), asthma (22.2%) and systemic allergy (11.4%).\(^6\) Family history of keratoconus has been reported to range from 6% to 20%\(^1\)\(^,\)\(^7\)\(^,\)\(^8\) and a predominantly autosomal dominant mode of inheritance is presumed, however, many researchers concur that the disease aetiology is multifactorial with both genetic and environmental elements coupled with variable expressivity.\(^9\)\(^-\)\(^11\)

The diagnosis of keratoconus is made on the basis of a combination of clinical signs and corneal topographic/tomographic signs. Classic clinical signs of keratoconus include corneal thinning, Fleisher’s ring, Vogt’s striae and Munson’s sign.\(^2\)\(^,\)\(^3\) Computerised videokeratography is a well established tool for identifying and monitoring subjects with keratoconus.\(^3\)\(^,\)\(^12\), Li \textit{et al} recently used a combination of computerised videokeratography data and clinical signs to produce a practical classification system for identifying early keratoconus.\(^13\)

The Orbscan II tomographer (Bausch and Lomb, Rochester, NY, USA) combines Placido disc and slit beam scanning technologies to analyse and quantify the anterior corneal curvature and enables accurate imaging of the anterior and posterior corneal elevation with wide-field measurement of corneal thickness.\(^12\)\(^,\)\(^14\)\(^,\)\(^15\) The Orbscan II tomographer has been used to
quantitatively document the progression of keratoconus, identifying increased apex elevation, displacement of the apex, and decreased thinnest point pachymetry during progression over a 2 year period. Several reports have identified topographic parameters important in distinguishing keratoconic from normal corneal morphologic features. These include: asymmetric bow tie astigmatism, posterior corneal elevation, local areas of increased surface power, central corneal keratometric steepening and inferior-superior dioptric asymmetry of the cornea. 3, 15, 17

Keratoconus is largely regarded as a bilateral but asymmetric disease, affecting one eye earlier and more severely in most cases. Reports of inter-eye asymmetry between the corneal curvatures of keratoconus have noted differences of 3.50-4.00 diopters (D), 18, 19 although the causes of this asymmetry have yet to be established.

The aim of this study was to investigate the tomographic features and explore associations between keratoconus risk factors and disease phenotype in a large hospital-based population with keratoconus. The analysis of such factors has not previously been performed in such a large population in New Zealand. These data are particularly interesting in the New Zealand context, as New Zealand appears to have a particularly high prevalence of keratoconus. However, since the majority of the New Zealand population are of Northern European ethnicity (67.6%), 20 these data also have relevance to our understanding of keratoconus globally.

**4.2 METHOD**

Review of a large database of keratoconic subjects analysed by Orbscan II tomography (Bausch and Lomb, Rochester, NY, USA) attending subspecialty cornea and external disease
Clinics conducted at the Department of Ophthalmology, Auckland District Health Board, in conjunction with the Department of Ophthalmology, University of Auckland, New Zealand. To exclude misdiagnoses, all subjects with a diagnosis of keratoconus were identified from the clinical database and the clinical data were reconfirmed in conjunction with a review of all relevant Orbscan II tomography maps by two experienced examiners (Charlotte Jordan and Richard Johnson), both licensed optometrists, with 5 and 17 years experience respectively, based primarily in a public hospital anterior segment service providing contact lens and other services to keratoconics.

Diagnostic criteria included tomographic maps with abnormal areas of central, inferior or superior steepening in conjunction with any of the following: oblique or non orthogonal astigmatism >1.5D, simulated keratometry greater than 47D, or central corneal thickness less than 500µm, as based on the Lim et al classification scheme for suspect keratoconics.21

Having assessed the corneal tomography and made the provisional diagnosis of keratoconus, the clinical notes were accessed for each patient and these included an assessment by a sub-specialist corneal surgeon in relation to the other features of keratoconus including; visible corneal thinning, prominent corneal nerves, Vogt’s striae, Munson’s sign, Fleisher’s ring, a scissor reflex on retinoscopy and an oil droplet reflex on direct ophthalmoscopy. Data were also collected in respect to age, gender, self-reported ethnicity and where available: personal and family ocular histories, reported atopic conditions, and history of eye rubbing (since these subjects attended specialist corneal and external disease clinics these latter data were generally recorded).

In all subjects the more severely affected eye was used for analysis based on the maximum keratometry data. Six quantitative Orbscan II tomographic parameters were analysed: 1. Simulated keratometry (Max and Min K), 2. mean power in the 3mm zone, 3. fixation pachymetry (defined as pachymetry along the instrument axis i.e. the centre of the Orbscan
map), 4. location of maximum dioptic power relative to fixation point (including meridian, radius, elevation and local pachymetry), and 5. anterior best fit sphere (BFS) in mm and 6. posterior BFS in mm. The position of the anterior and posterior apex was utilised solely in terms of position in relation to x-y coordinates.

The simulated keratometry values were obtained from Orbscan maps. Colour-coded maps were examined using absolute scales in steps of 1D for axial power maps, 0.005mm for elevation data and in steps of 20µm for the pachymetry maps (with an acoustic factor of 0.92). The anterior and posterior elevations were determined by the recorded maximum elevation relative to the best-fit sphere. The current study used the Orbscan default best-fit sphere as this provides the best standardised and repeatable measure at a single time point and allows comparisons with other published studies assessing elevation using the Orbscan system.  

Keratoconic eyes were further classified by severity, based on simulated maximum keratometric value. Severity classifications were based on the Collaborative Longitudinal Evaluation of Keratoconus (CLEK) guidelines, where a maximum simulated keratometry of <45D is classified as mild keratoconus, 45 - 52D moderate keratoconus, and >52D severe keratoconus. There is no widely accepted severity classification system for keratoconus based on anterior or posterior elevation or wide-field pachymetry maps, therefore to enable ready comparison with other published studies the current study has utilised the CLEK study topography classification system.
Figure 4-1: Classification maps for axial map morphology of the most severely affected eye (n = 266) (after Rabinowitz et al.\textsuperscript{25}).

Eyes were also classified by axial map morphology based on the Rabinowitz classification system (Figure 4-1).\textsuperscript{25, 26} Axial maps were classified into one of ten subgroups: round, oval, superior steepening, inferior steepening, and irregular symmetrical, symmetrical with skewed axis, asymmetric with inferior steepening, asymmetric with superior steepening and asymmetric with skewed axis.
Elevation map morphology was classified based on the system reported by Naufal et al (Figure 4-2). To determine symmetry or enantiomorphism, only subjects with bilateral tomography data were used.

The ethnicity statistics for the overall population of patients attending the tertiary Ophthalmology facility, within which the cornea and external disease clinics are based, were obtained from the Clinical Data Analyst, Auckland District Health Board.

Any patient whose clinical documents could not be accessed, or any patient misdiagnosed based on corneal tomography maps, was excluded. Maps were also excluded if the associated clinical features may have created artefacts and thus made them potentially inaccurate in terms of classification (e.g. corneal scarring, concurrent ocular surface disease,
prior surgery or rigid contact lens wear immediately prior to the tomography acquisition).

Orbscan tomographic maps were excluded from analysis if; the majority of the central 6mm of the scan had missing data, if the scan was too irregular for adequate analysis, if any map did not include all four descriptive maps provided by the Orbscan system (axial map, pachymetry map, and the anterior and posterior elevation maps), or where obvious artefacts were present (e.g. where very high keratometric values (>70D) were present.)

The study design adhered to the tenets of the Declaration of Helsinki with Institutional Research Ethics Board approval.

4.3 STATISTICAL ANALYSIS

The statistical programme SAS (Statistical Analysis System Version 9.1, SAS Institute Inc. Cary, NY, USA) was utilised to undertake multifactorial analysis. In order to assess variables that are associated with individual characteristics of keratoconus, separate general linear models were fitted for the outcomes of severity (Max K), mean power 3mm zone, fixation pachymetry, thinnest pachymetry, location of maximum dioptic power relative to fixation point (including meridian, radius, elevation and local pachymetry). The x, y coordinates of the positions of the thinnest point, and anterior and posterior apex were also used as multivariate outcomes.

Explanatory variables included in all analyses were presence of allergies, a family history of keratoconus, history of eye rubbing, age, gender and ethnicity.

Comparison of axial map classification systems incorporated explanatory factors using One-way ANOVA with Tukey’s multiple comparison post-hoc test. Statistical significance was defined as p<0.05.
4.4 RESULTS

A total of 601 eyes of 353 subjects were identified with a diagnosis of keratoconus over a four year period. Eighty seven subjects were excluded due to excessive scarring (hydrops), concurrent RGP wear or previous ocular surgery. Therefore, the final analyses included 532 eyes of 266 subjects. The study population consisted of 122 females (46%) and 144 males (54%). The mean age was 29.3 ± 11.6 (mean ± standard deviation) (Range 10-79) years.

For the purpose of analysis in relation to risk factors and severity, only the more severely affected eye of each patient was selected. Of these 266 subjects, 144 (54%) had complete Orbscan II data available for both eyes and these were also included in the analysis of enantiomorphism.

4.4.1 Ethnicity

Self reported rates of ethnicity in keratoconic subjects compared to the overall population of patients attending the Ophthalmology Department, Auckland District Health Board, are shown in Table 4-1. The keratoconic group had significantly higher proportions of Maori and Pacific subjects and lower rates of European and Asian subjects when compared to the total population (p = 0.0001).

Asian (21%), Pacific (17.7%) and Maori (13.3%) populations had significantly higher rates of familial history of the disease, compared to the European population (3.5%) (Table 4-2).
Table 4-1: Distribution of self-reported ethnicity in the keratoconic population (N=266) compared to the tertiary corneal clinic, total ophthalmology clinic, and total New Zealand populations, highlighting over-representation of patients of Pacific and Maori ethnicity (p<0.001). *Self-reported data obtained from National census; therefore some subjects identified themselves with more than one ethnicity

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Keratoconus (%)</th>
<th>Tertiary Corneal Clinic Population (%)</th>
<th>Total Ophthalmology Clinic Population (%)</th>
<th>Total New Zealand Population *(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>European</td>
<td>28.4</td>
<td>48.4</td>
<td>53.3</td>
<td>67.6</td>
</tr>
<tr>
<td>Pacific Peoples</td>
<td>33.1</td>
<td>16.3</td>
<td>12.5</td>
<td>14.6</td>
</tr>
<tr>
<td>Maori</td>
<td>18.4</td>
<td>8.6</td>
<td>6.5</td>
<td>9.2</td>
</tr>
<tr>
<td>Asian</td>
<td>6.6</td>
<td>13.1</td>
<td>15.3</td>
<td>6.9</td>
</tr>
<tr>
<td>Other</td>
<td>8.0</td>
<td>7.0</td>
<td>6.9</td>
<td>10.6</td>
</tr>
<tr>
<td>Not Stated</td>
<td>5.5</td>
<td>6.5</td>
<td>5.6</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4-2: Rates of self-reported family history of keratoconus according to ethnicity.

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>No. with family history</th>
<th>Total No. subjects</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>European</td>
<td>3</td>
<td>84</td>
<td>3.5</td>
</tr>
<tr>
<td>Maori</td>
<td>6</td>
<td>45</td>
<td>13.3</td>
</tr>
<tr>
<td>Pacific</td>
<td>17</td>
<td>96</td>
<td>17.7</td>
</tr>
<tr>
<td>Asian</td>
<td>4</td>
<td>19</td>
<td>21</td>
</tr>
</tbody>
</table>
4.4.2 Severity Classification

The majority of the eyes were classified into the severe category 155 (58.3%), with 90 (33.8%) identified as moderate and 21 (7.2%) as mild (Table 4-3).

The mean thinnest pachymetry differed significantly between the mild (436 ± 60 µm) moderate (414 ± 52 µm) and severe (385± 65µm) keratoconus groups (p<0.001 one way ANOVA). When analysing the data overall, significant negative correlations were observed between severity of keratoconus and; thinnest point pachymetry ($r= -0.57$, $p<0.0001$), fixation pachymetry ($r= -0.465$, $p<0.0001$), and apical pachymetry ($r= -0.645$, $p<0.0001$). However, there was no significant correlation between keratoconus severity and anterior apical elevation ($r= -0.096$, $p=0.1053$), or posterior apical elevation ($r= -0.061$, $p=0.30$). No significant association was identified between disease severity and a self-reported history of eye rubbing ($p=0.65$). This interaction was therefore removed from subsequent analysis.

<table>
<thead>
<tr>
<th>Keratoconus Severity Classification</th>
<th>Number of eyes</th>
<th>Percentage of eyes</th>
<th>Median Max K (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild (&lt;45D)</td>
<td>21</td>
<td>7.8</td>
<td>43.9</td>
</tr>
<tr>
<td>Moderate (45-52D)</td>
<td>90</td>
<td>33.8</td>
<td>48.4</td>
</tr>
<tr>
<td>Severe (&gt;52D)</td>
<td>155</td>
<td>58.3</td>
<td>57.8</td>
</tr>
</tbody>
</table>

Table 4-3: Severity of keratoconus based on simulated keratometry, with median maximum keratometry values, in 266 eyes with keratoconus. (based on CLEK guidelines.23)

There was no significant relationship between disease severity and patient age ($p=0.50$), patient gender $p= (0.19)$, ethnicity ($p=0.19$) or the presence of concurrent allergies ($p=0.43$).
Thirty six subjects (12.4%) reported a family history of keratoconus and this was the only variable that demonstrated strong evidence of an association with severity of keratoconus (p=0.006), with a positive family history being indicative of less severe disease.

When compared to subjects without a family history of keratoconus, a positive family history of keratoconus was associated with a significantly lower mean power in the 3mm zone (p=0.01) and a significantly greater thinnest point pachymetry (p=0.03).

4.4.3 Atopy, Age and Gender

Seventy four subjects (27.8%) had a history of ocular allergy, 76 subjects (28.6%) were asthmatic and 65 subjects (24.4%) had eczema (Table 4-4). Twelve subjects reported a history of ocular allergy, asthma and eczema (4.5%). One hundred and four subjects (36%) reported a history of eye rubbing (Table 4-4). Of these subjects 32 (12.03%) had a sole risk factor of ocular allergy, 19 (7.1%) had the sole risk factor of asthma and 15 (5.6%) had the sole risk factor of eczema. There was no significant association between age, gender, ethnicity and the presence of allergy and eye rubbing and the topographic parameters investigated (Table 4-5).

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Percentage of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopic history:</td>
<td></td>
</tr>
<tr>
<td>Ocular Allergy</td>
<td>27.8</td>
</tr>
<tr>
<td>Eczema</td>
<td>24.4</td>
</tr>
<tr>
<td>Asthma</td>
<td>28.6</td>
</tr>
<tr>
<td>Eye Rubbing</td>
<td>36.0</td>
</tr>
<tr>
<td>Family History of Keratoconus</td>
<td>12.5</td>
</tr>
</tbody>
</table>

*Table 4-4: Rates of self-reported atopy, eye rubbing and family history of keratoconus (N=266).*
4.4.4: Aetiological Risk Factors and Tomographic Classification

When comparing statistical outcomes from subjects who were reported to have only a family history (FH) without other potential risk factors, and subjects with only atopy, there were statistically significant differences between the mean thinnest pachymetry (FH 340±15 µm, atopy 381 ±8 µm; p= 0.0218) and apical pachymetry (FH 355 ±18 µm, atopy 401 ±15 µm; p= 0.0071). There was also a statistically significant difference in thinnest pachymetry between subjects with and without a family history of keratoconus (p=0.03) (Table 4-5).

4.4.5 Axial Map Morphology

The most common morphology identified was the asymmetric bowtie pattern with inferior steepening in 81 eyes (29%). The next most common morphologies were round 51 (18%) and inferior steepening 46 (17%) (Figure 4-1). An inter-observer Cronbach’s Alpha of 0.996, indicated good classification repeatability between the observers.

4.4.6 Anterior Elevation Map Morphology

The most common anterior elevation map classification was that of the spur morphology 138 (49.3%) maps. This was followed in decreasing frequency by island 67 (24%), irregular ridge 42 (15%), regular ridge 18 (6%), unclassifiable 13 (4.6%) and incomplete ridge 2 (0.7%), map classification (Figure 4-2). An inter-observer Cronbach’s Alpha of 0.989 indicated good classification repeatability between the observers. Anterior elevation maps were classified as spur (49.3%), island (24%), irregular ridge (15%) or other (11.3%).
### Table 4-5: Topographic parameters of the differing subgroups based in potential risk factors of a) family history b) eye rubbing and c) atopy. * Statistically significant

#### 4.4.7 Posterior Elevation Map Morphology

Posterior elevation maps were classified as island morphology 121 (43.2%), spur 106 (37.9%), irregular ridge 23 (8%), unclassifiable 25 (8.9%), regular ridge 3 (1%) and incomplete ridge 2 (0.7%) (Figure 4-2). An inter-observer Cronbach’s Alpha of 0.969 indicated good classification repeatability between the observers.
4.4.8 Enantiomorphism

In relation to the symmetry of the anterior BFS, posterior BFS and axial keratometric map morphologies, of the 144 subjects analysed, 33 (22.9%) had 2 of 3 identical classifications between the right and left eyes. Eighteen (12.5%) subjects had complete enantiomorphism with 3 of 3 identical classifications.

4.5 DISCUSSION

Although many theories exist regarding the aetiology of keratoconus, none of these have yet proven conclusive. Commonly cited aetiological factors include hereditary, systemic and atopic disease, and mechanical trauma due to eye rubbing. However, the multi-factorial nature of keratoconus and the effect of environmental influence on disease phenotype remain to be fully elucidated.

Armitage et al investigated possible aetiological factors in relation to cone morphology, and using a questionnaire based method, they assessed age of onset, gender, atopic history and the number of family members with keratoconus. However, they identified no link between any of the potential aetiological factors and the morphologic features. Others have also failed to identify differences in keratoconic histopathology in respect to patient demographics in those undergoing corneal transplantation.

Inheritance of keratoconus is largely thought to be autosomal dominant with variable expressivity. The current hospital clinic based study established a familial rate of keratoconus of 12.4% in this New Zealand population, which generally corresponds with internationally reported rates of 6% to 20%. Anecdotally, the prevalence of keratoconus in New Zealand is thought to be higher than reported estimates in other countries. It has also long been postulated that Maori and Pacific populations have a higher incidence of
keratoconus than other populations in New Zealand and the current study tends to support this observation. Such population differences may suggest varying genetic influences within these ethnic groups and this may be reflected by the higher rates of family history occurring in the Maori and Pacific, compared to the European, keratoconic sub-groups. However, in terms of ethnicity, one limitation of this study is that the keratoconic group were compared to a large overall population attending an ophthalmology service, rather than an age matched general population.

This study elucidated phenotypic differences between subjects with, and those without, a family history of keratoconus. There were significant differences between these groups in terms of severity of disease, mean keratometric power (3mm zone) and thinnest pachymetry. Interestingly, disease severity in subjects with a family history was actually less than those without a family history. This may indicate differences in disease processes within different subgroups or an earlier presentation of those with a family history. Interestingly, the CLEK study investigated disease severity in subjects with a family history of keratoconus using univariate analysis and noted no significant relationship. However, in this more severely affected population, using multivariate analysis we were able to exclude the influence of exogenous variables to establish relationships.

An association between keratoconus and atopy is long established, though whether this is a coincidental or a causal relationship is still debated. A higher incidence of eczema has been reported in keratoconic subjects and higher rates of atopic disease (eczema, asthma and hayfever) were noted in those keratoconics presenting to Moorfields Eye Hospital, London. The Dundee University Scottish Keratoconus Study (DUSKS) noted self reported rates of asthma, eczema and hayfever of 23%, 14% and 30% respectively. In the current study, 26.2% of keratoconics had asthma, 22.4% had eczema and 25.5% of reported ocular allergy. Although these results are similar to those of the DUSKS study, and notably the majority of the New Zealand European population originate from the United Kingdom, the current study
noted many more subjects with eczema in the New Zealand cohort. In comparison the North America based CLEK study noted significantly higher rates of systemic allergy (52.9%), however, the authors reported lower rates of asthma and eczema. Notably, the incidence of eczema and asthma in the current study were similar to those observed by Owens and Gamble in an earlier New Zealand study of Keratoconus, where they reported 34% exhibited asthma and 25% had eczema. These data, in conjunction with the present study, may lead us to postulate that the higher rates of keratoconus may be, directly or indirectly, related to the higher rates of atopic disease within New Zealand populations.

In the current study, there was no significant difference in tomographic phenotype between those subjects with or without ocular allergy. In contrast, Kaya et al reported significant tomographic differences between subjects with atopy and those without, in respect to a number of measures including: mean central and thinnest corneal pachymetry, distance of thinnest point to corneal centre, anterior and posterior elevation values, and irregularity indices. Whilst this study suggested phenotypic variations associated with atopic disease and therefore potential causal disease mechanisms, our study did not identify similar trends. However, the latter may reflect differences in data collection between the study groups, as the current study relied on retrospective review of a hospital based population with relatively advanced disease.

The current study reported significant differences in the tomographic characteristics of subjects with an isolated family history of keratoconus compared to those without a family history but with a history of any ocular allergy. These included differences in thinnest pachymetry, and fixation pachymetry. Therefore in this study those subjects with ocular allergy as their only aetiological risk factor tended to have less severe disease than those with a family history of keratoconus. This must be considered in the context that, overall; those subjects with a family history of keratoconus (12.4%) had less severe tomographic features of keratoconus than the remainder of the study population. Selection bias may have influenced
these results, since family members of affected patients may be more likely to seek disease screening at an earlier stage compared to those without a family history.

Chronic, low grade, corneal trauma caused by eye rubbing has long been postulated in the pathogenesis of keratoconus. It has been reported that following epithelial wounding that increased levels of interleukin-1 result in significant keratocyte apoptosis and subsequent loss of stromal volume. It has also been postulated that intrinsic abnormalities in enzyme function, present in keratoconic corneas, combined with the trauma of eye rubbing result in apoptosis of keratocytes in the anterior stroma with subsequent loss of stromal homeostasis. In this context, alteration in expression of apoptotic genes, increase in keratocyte IL-1 receptors and a decrease in keratocyte density have all been implicated in the pathogenesis of keratoconus.

The cause and effect relationship between eye-rubbing, atopy and keratoconus is not clear. It has been reported that atopic traits are more common in patients with keratoconus than in general ophthalmic patients. However, it has been suggested that allergy, itch and eye rubbing are only relevant in the pathogenesis of keratoconus when the highest levels of these factors are present. The cyclical nature of this relationship cannot be ignored, with atopic disease often leading to eye rubbing and therefore the mechanical trauma mentioned earlier. Also we must consider whether the eye rubbing is simply the influence needed to move a subject with a genetic predisposition into a diseased state. Further associations between systemic diseases and both keratoconus and eye rubbing, have also been identified. For example, Leber congenital amaurosis is associated with keratoconus and the disease is also associated with persistent eye rubbing from the oculo-digital response.

The current study observed no significant difference between the tomographic maps of subjects with self-reported eye rubbing and those without a history of eye rubbing. In view of the retrospective nature of this study we cannot definitely exclude phenotypic differences that
may occur due to eye rubbing, since this may have been under-reported. Indeed, the current study had a significantly lower proportion of subjects with a history of eye rubbing than similar international studies.  

Liu et al investigated the morphology patterns of 46 normal corneas and observed that 71.8% of the anterior corneal elevation maps and 32.6% of posterior elevation maps were classified as island. In terms of axial keratometric map morphology they noted that symmetric bow tie was the more common keratoconic morphology. Keratoconus is often asymmetrical in disease severity between a subject’s eyes, although the topographic morphology is thought to be more symmetrical in nature when analysing videokeratography. However, in the current study only 12.5% of keratoconic subjects had complete mirror symmetry of tomographic morphology between the eyes. This suggests that the disease is not only asymmetric in severity, but also in morphological pattern within an individual.

Limitations of our study include the fact that inclusion of those with data only from one available eye may alter our results, as presumably many of those who had undergone contralateral penetrating keratoplasty originally had more severe disease in that eye. The retrospective design of the study also relied upon accurate clinical documentation at the patient’s earliest clinic examination. Although it is difficult to accurately assess the completeness of the data acquired from the chart review, all patients were reviewed in a cornea and anterior segment clinic with a reasonably standardised approached to clinical documentation.

The exclusion of patients in whom corneal tomography maps were either unavailable or unreliable may have an impact on the associations observed between corneal phenotype and aetiological factors. Future studies using corneal imaging technology may enhance our understanding of the corneal phenotype in keratoconus by enabling analysis of additional parameters such as corneal volume.
The results of this study indicate that in a large tertiary population with advanced keratoconus tomographic phenotypic variations occur in subjects with differing aetiological risk factors. This study specifically identifies phenotypic differences occurring in subjects with, compared to those without, a family history. The results also suggest overrepresentation of Maori and Pacific ethnicities in the New Zealand keratoconic population. This study identified largely asymmetric corneal disease as assessed by current tomographic classification systems.

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**Jordan CA, Zamri A, Wheeldon C, Patel DV, Johnson R, McGhee CN.**

REFERENCES


SECTION III

Ocular Response Analyser Studies

(Chapters 5 – 6)
Chapter 5: Repeatability and Variability of Intraocular Pressure and Corneal Biomechanical Properties as Measured by the Ocular Response Analyser
5.1 INTRODUCTION

The Ocular Response Analyser (ORA; Reitchert Ophthalmic Instruments Inc., Depew, New York, USA) measures intraocular pressure (IOP) and biomechanical properties of the cornea in vivo using the principles described in Chapter 2.  

The ORA has been postulated as an adjunctive clinical tool for diagnosing and monitoring several ocular diseases e.g. keratoconus  

and Fuchs' corneal endothelial dystrophy. Both of which have been shown to have significantly lower Corneal Hysteresis (CH) compared to normal corneas. Corneal Hysteresis and Corneal Resistance Factor (CRF) have also been shown to decrease after laser in situ keratomileusis (LASIK). It has been suggested that the ORA may be a useful tool in pre-operative assessment for keratorefractive surgery, potentially identifying forme fruste keratoconus.

Accurate measurement of intraocular pressure IOP is crucial in the diagnosis and management of glaucoma and for several decades Goldmann applanation tonometry has been regarded as the gold standard method of measuring IOP. Corneal thickness is known to influence IOP measurement. The Goldman tonometer assumes a corneal thickness of 500µm and therefore tends to underestimate IOP in thinner corneas whilst overestimating IOP in thicker corneas. It has been suggested that the ORA provides a more accurate method for measuring IOP by providing measurements that are not affected by central corneal thickness.

Reports have also suggested that the ORA may provide more reliable day to day IOP measurements when compared to Goldmann and non-contact tonometry; although repeatability indices for the ORA are actually poorer than those with the Goldmann tonometer.
single examination have noted highly reproducible IOP$_{G}$ and IOP$_{CC}$ values, provided a mean of at least three measurements are analysed.\footnote{11}

The aims of this ORA based study were a) to evaluate the diurnal variation in IOP and corneal biomechanical properties b) to evaluate the day to day variation in IOP and corneal biomechanical properties c) to determine any relationship between diurnal variation of IOP, CH and CRF, d) to assess the intra-observer repeatability of ORA measurements.

5.2 METHODS

A pilot study on diurnal variation was carried out on 3 subjects (mean age 41.3 years ± 3.2 years SD) to enable an accurate sample size power calculation. The right eye of each subject was tested hourly from 0900 to 1600 during one working day. At each hourly measurement 3 repeated readings were taken with waveform scores of 5.0 or above. The reading with the highest waveform score was used for sample size analysis. To obtain 80% power to detect a real change of 1mmHg over the 7 hour period at the 5% level of significance required a minimum number of 20 subjects for CRF and 15 for CH. For the study to detect a difference of 1mmHg with 90% power the required sample size was 25 subjects. The final sample size was determined to be 34 for diurnal variation, 27 for day to day variation and 51 for repeatability measurements.

Healthy participants were recruited from staff of the Department of Ophthalmology, Auckland District Health Board, Auckland, New Zealand and their friends and relatives. Exclusion criteria included any previous ocular injury, ocular surgery, current contact lens wear or previously diagnosed ocular pathology of either eye or systemic disease that may affect the cornea. The study design adhered to the tenets of the Declaration of Helsinki with Institutional Research Ethics Board approval. Informed consent was obtained from all subjects after providing written and verbal information regarding the study and the procedures involved.
One eye of each participant was examined and the eye was selected by random number allocation using random number tables. All eyes were examined using the ORA as previously described in chapter 2.

Diurnal variation measurements were obtained at hourly intervals between the hours of 8:30 am to 5:30 pm and the time at which each subject awoke was recorded. Inter-day variation measurements were obtained at the same time of day over 5 consecutive days. Data were excluded for any day where a subject was unable to attend a visit within an hour of the initial visit time.

Three consecutive measurements were obtained on all subjects for every time point (both diurnal and day to day). Five quantitative parameters were analysed using the ORA:

1. Goldmann correlated intraocular pressure ($\text{IOP}_G$)
2. Corneal compensated intraocular pressure ($\text{IOP}_{CC}$)
3. Corneal hysteresis (CH)
4. Corneal Resistance factor (CRF)
5. The waveform score simulated by the ORA

The waveform score provides an indication of the accuracy and reliability of the ORA measurement. The waveform score is presented on a scale of 0 to 10; for the current study; only signals with a waveform score of 5 or above were accepted.
5.3 STATISTICAL METHODS

Reliability measures were analysed using SPSS 15.0 for Windows data software (SPSS Inc Chicago, Illinois, USA). The internal consistency of the observations was measured by calculating the intra class correlation coefficient (ICC) for the day to day variability. Repeated measures ANOVA analysis was performed on each time group to establish if equal means existed. Repeated measures ANOVA analysis was used to establish any differences between the diurnal measurements between subjects. Statistical significance was identified as p <0.05.

5.4 RESULTS

One eye of 34 normal subjects (mean age 39.2 ± 18.2 yrs) was examined for the purposes of the diurnal variation study. To identify day to day variation one eye of 28 normal subjects (mean age 40.9 ±10.5 yrs) was examined.

5.4.1 Diurnal Variation

The mean diurnal variation in IOP_G and IOP_CC between 8:30 am to 5:30 pm was 2.8 ± 1.4 mmHg and 3.9 ± 5.0 mmHg respectively. Repeated measures ANOVA analysis revealed no statistically significant variation in IOP_G (p=0.49) or IOP_CC (p=0.77) throughout the day.

The mean difference between IOP_CC and IOP_G was 1.1 ± 0.1 mmHg (p=0.01) and the highest IOP values occurred in the morning, 1-2 hours after awakening. (Figure 5-1)
Figure 5.1: Mean IOP$_G$, IOP$_{CC}$, CRF and CH measurements recorded from 1-12 hours from awakening demonstrating diurnal variation.

The mean diurnal variations in CRF and CH were 0.8 ± 1.3 mmHg and 1.7 ± 2.6 mmHg respectively. Repeated measures ANOVA analysis revealed no significant variation in CRF (p=0.48) or CH (p=0.79) across the day. The lowest CH and CRF values occurred in the morning, 1-2 hours after awakening (Figure 5-1).

A moderately significant correlation was noted between the diurnal variation of IOP and CRF (Figure 5-2). The correlation was stronger for IOP$_G$ than for IOP$_{CC}$ ($r^2 = 0.54$ p=0.01 and $r^2 = 0.42$ p=0.03, respectively). There was no significant correlation between CH and either IOP$_G$ or IOP$_{CC}$ ($r^2 = 0.25$ p=0.11 and $r^2 = 0.37$, p=0.4 respectively) (Figure 5-3).
Figure 5.12: Correlation of the variation of $IOP_g$ and $IOP_{cc}$ with variation of CRF.
5.4.2 Day to Day Variation

There was no significant difference in the mean IOP$_G$ (1.2 ± 1.7 mmHg, $p=0.40$), IOP$_{CC}$ (1.1 ± 1.8 mmHg, $p=0.36$) and CRF (0.4 ± 0.5 mmHg, $p=0.16$) and CH (0.3 ± 0.4 mmHg, $p=0.10$) between any of the consecutive daily measurements.

Intra class correlation coefficient values for IOP$_G$ (0.93, 95% CI 0.87 - 0.97), IOP$_{CC}$ (0.90, 95% CI 0.80 - 0.95) and CRF (0.95, 95% CI 0.90-0.98) and CH (0.88, 95% CI 0.77 - 0.95), revealed clinically acceptable levels of repeatability from day to day.

The mean CH was significantly higher than the mean CRF for each day ($p=0.01$) (Figure 5-4).
Figure 5.14: Day to day variation of mean IOP$_g$, IOPcc, CRF and CH.
5.4.3 Repeatability

Repeatability data consisted of 120 measurements on 51 eyes. Each measurement consisted of three repeated readings taken within 4 minutes of each other with waveform scores of 5 or above.

Analysis of waveform scores of the 120 repeated measurements showed that the mean waveform scores of the first, second and third readings were 7.2, 7.3 and 7.3, respectively. Figure 5-5 shows the repeatability of the measured parameters with 95% confidence intervals highlighted in Table 5-1.

![Graphs showing repeatability of IOPg, IOPcc, CRF and CH for waveform scores of 5-10]

**Figure 5.15:** Repeatability of IOP_{G}, IOP_{CC}, CRF and CH for waveform scores of 5-10 showing the distribution of the mean difference.
There is growing interest in the biomechanical properties of the cornea among both clinicians and researchers. Previously there has been no simple method to determine corneal biomechanical properties \textit{in vivo} and clinicians were limited to measuring CCT as an indirect measure of ocular rigidity.\textsuperscript{13} The ORA was recently introduced as the first \textit{in vivo} technique for measuring corneal biomechanical properties (see chapter 2).

The cornea is a complex viscoelastic tissue which produces a time dependent corneal strain response when placed under stress. The inward applanation reading measured by the ORA is purported to be a result of the immediate response of the elastic properties of collagen fibres in the cornea, whereas the outward applanation is believed to be due to the delayed response of elastic properties of the corneal matrix.\textsuperscript{13} Stable inter-observer and diurnal ORA measures of corneal biomechanical properties have previously been reported.\textsuperscript{14-17, 18, 19} Interestingly, the only measure that showed variability in the ORA parameters was IOP, ranging from the

<table>
<thead>
<tr>
<th></th>
<th>Repeatability Range of ORA measures (95% confidence) (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IOP(_G)</td>
<td>-1.9 to 2.7</td>
</tr>
<tr>
<td>IOP(_{CC})</td>
<td>-2.4 to 3.7</td>
</tr>
<tr>
<td>CRF</td>
<td>-2.4 to 1.3</td>
</tr>
<tr>
<td>CH</td>
<td>-1.9 to 1.3</td>
</tr>
</tbody>
</table>

\textbf{Table 5.1:} Repeatability ranges of IOP\(_G\), IOP\(_{CC}\), CRF and CH with 95% confidence intervals

### 5.5 DISCUSSION

The cornea is a complex viscoelastic tissue which produces a time dependent corneal strain response when placed under stress. The inward applanation reading measured by the ORA is purported to be a result of the immediate response of the elastic properties of collagen fibres in the cornea, whereas the outward applanation is believed to be due to the delayed response of elastic properties of the corneal matrix.\textsuperscript{13} Stable inter-observer and diurnal ORA measures of corneal biomechanical properties have previously been reported.\textsuperscript{14-17, 18, 19} Interestingly, the only measure that showed variability in the ORA parameters was IOP, ranging from the
highest measures in the morning to the lowest in the evening.\textsuperscript{18, 19} The diurnal variation of IOP is a well-recognised phenomenon in both normal\textsuperscript{20-22} and glaucomatous eyes.\textsuperscript{22, 23, 24} Diurnal fluctuations in IOP have been reported to range from 3.2 - 6.5 mmHg in normal eyes\textsuperscript{20-22} and a somewhat greater range of 11.0 - 16.0 mmHg recorded in glaucomatous eyes.\textsuperscript{21, 24} The peak IOP was noted to occur in the early hours of the morning (6:00 - 9:00am) in healthy subjects.\textsuperscript{21, 25}

In the current study the mean diurnal variation in \(\text{IOP}_G\) and \(\text{IOP}_{cc}\) was 2.8 ± 1.4 mmHg and 3.9 ± 5.0 mmHg respectively between 8:30am and 5:30pm, neither measure however was statistically significant. This lack of statistical significance may be partly explained by the timings of measurements used for this study in that the highest early morning IOP peak may have been missed in some individuals. The highest IOP measurements recorded occurred 1-2 hours after waking followed by a stable profile up to 5:30 pm (up to 12 hours post waking). However we must consider the large standard deviation noted in the diurnal variation of \(\text{IOP}_{cc}\) to be clinically significant when examining an individual. This large standard deviation would suggest significant fluctuations in the IOP measurements over the period of a day.

The diurnal IOP profile obtained in this study is in agreement with the majority of other published studies.\textsuperscript{14, 26} Other studies have reported significant diurnal variations in IOP measured with the ORA.\textsuperscript{13, 27, 28} Indeed, two studies showed very significant variations in IOP but these measured IOP over an entire 24 hour period compared to the 9 hour period of the current study.\textsuperscript{19, 28} This strategy may have resulted in higher peak IOP measurements associated with the supine position of patients during sleeping hours (which is known to increase IOP).\textsuperscript{29} One other study variation used topical anaesthetic prior to IOP measurement and so may have affected IOP measurement by altering CCT.\textsuperscript{18}

The current study endeavoured to identify relationships between the biomechanical properties of the cornea and measured IOP. A modest correlation between the diurnal variation of IOP
and CRF was observed, with the correlation being stronger for \( \text{IOP}_G \) than for \( \text{IOP}_{CC} \) (\( r^2 = 0.54, p=0.01 \) and \( r^2 = 0.42, p=0.03 \), respectively). Central corneal thickness may be responsible for this relationship as CCT varies during the day and this is known to affect Goldmann tonometry. \(^{28,30}\) The corneal resistance factor has been reported to be more indicative of the mechanical strength of the cornea irrespective of corneal thickness, however, moderate correlations between CCT and both CH and CRF have previously been reported. \(^2\)

In the current study there was no significant diurnal variation in CH or CRF between 8:30 am and 5:30 pm. These results are also in agreement with previous reports of stable profiles of CH and CRF with no significant variation during “working” hours. \(^{13,26,27,31}\) However, during a 24 hour cycle CRF has been shown to increase immediately after awakening when compared with a baseline value taken before sleep at 10:00 pm. \(^{28}\) In contrast CH remained stable throughout the 24 hour period. \(^{28}\)

The relationships between CCT and all the ORA measures except \( \text{IOP}_{CC} \) have been previously reported and we have not explored these further. \(^5,13,27,32,33\) The current study is the first to show no statistically significant day to day variation in CH and CRF and would indicate that the ORA provides repeatable measures that may be compared inter-day. In the context of the cross-linking component of the reported studies this data provides reassurance about the quality of our measured parameters.

In the current study, there was no significant correlation between \( \text{IOP}_{CC} \), CH and CRF; therefore \( \text{IOP}_{CC} \) varies independently of both factors. This result is consistent with that of Laiquzzaman et al. \(^{13}\) and is in general agreement with the manufacturer’s claims that the \( \text{IOP}_{CC} \) provides pressures independent of variation in corneal structure. Other studies have noted a significant but weak correlation between \( \text{IOP}_{CC} \) and CH noting that the \( \text{IOP}_{CC} \) tended to increase as the CH increased. \(^5\) Shen et al. \(^{34}\) also reported a correlation between
increases of $IOP_G$ and $IOP_{cc}$ with an increase in CRF but this study was carried out over a 24 hour period where significant diurnal variations of IOP and CRF were detected. 34

More accurate IOP measurements through $IOP_{cc}$ may prove beneficial in the diagnosis and treatment of glaucoma as well reducing unnecessary wrong diagnosis and treatment. As suggested by Moreno-Montanes et al. 35 CH could ultimately be considered as a pressure-independent risk factor for glaucoma as it does not show significant diurnal variation and lower CH values were associated with visual field loss in glaucoma. 33 Further research to determine the diurnal variation of CH and CRF may improve diagnostic accuracy in corneal pathologies such as keratoconus.

The current study demonstrates that the ORA produced repeatable measurements for readings with waveform scores of 5 or above. It was also observed that good intra-examiner reproducibility with all ORA parameters was achieved with waveform scores of 5-10.

Identifying the repeatability and reliability of an instrument is essential for researchers and clinicians. The current study has shown that the ORA provides repeatable and reliable measures of both the corneal biomechanics and IOP between the hours of 8:30am and 5pm, and between inter-day measurements. Therefore, the intra and inter-day measurements are not a significant variable when using the ORA in the clinical or research setting; as I do hereafter in this series of studies on keratoconus and collagen cross-linking.
REFERENCES


Chapter 6: Corneal Hysteresis and Corneal Resistance Factor in Keratoconus
6.1 INTRODUCTION

As previously highlighted keratoconus is a non-inflammatory disorder in which the cornea assumes a conical shape subsequent to thinning and protrusion. Clinically, corneal ectasia leads to myopia and irregular astigmatism. In severe cases rupture in Descemet’s membrane may occur resulting in corneal oedema and scarring. The ability to distinguish early keratoconic changes is particularly important especially considering the increasing popularity of refractive surgery for myopia. As outlined in detail in Chapter 1 the diagnosis of keratoconus is usually made on the basis of a combination of clinical and corneal topographic signs. Mild cases of keratoconus however are difficult to detect clinically; thus several topographic parameters have been developed to distinguish keratoconic from normal corneas. These parameters include asymmetric bow tie astigmatism, posterior corneal elevation, local areas of increased surface power, central corneal keratometric steepening and inferior-superior dioptric asymmetry.

Topographic indices have been shown to have a high degree of sensitivity and specificity in the detection of keratoconus. Diagnosing forme fruste keratoconus remains challenging especially considering that the diagnostic indices derived from Placido disc based systems only use data from the anterior corneal surface. More recently the development of corneal tomography has enabled additional tomographic analysis of the posterior corneal surface. Posterior corneal elevation has been shown to be particularly useful in discriminating keratoconus from normal corneas. However, it is insufficient as a single parameter alone and should be combined with corneal curvature data to optimise diagnostic value.

The biomechanical strength of the cornea is thought to be reduced in keratoconus. Unfortunately the ability to measure the corneal strength in vivo has previously been limited to extrapolation from corneal thickness measurements. The introduction of the Ocular Response
Analyser (ORA, Reichert, NY, USA) has led to a dramatic increase in research investigating the intrinsic biomechanical properties of the cornea and the subsequent effect of corneal disease and surgical procedures (see Chapters 2 and 5). The ORA has been promoted as an adjunctive tool in the diagnosis of keratoconus and is reported to distinguish mild and severe keratoconus but it cannot reliably discriminate mild keratoconus from normal corneas. 13, 14-16

The aim of this study was to determine the relationship between ORA measured corneal biomechanical properties specifically Corneal Resistance Factor (CRF), Corneal Hysteresis (CH) and posterior corneal elevation.

### 6.2 METHODS

A prospective analysis was performed on patients with keratoconus recruited from the Department of Ophthalmology, Greenlane Clinical Centre, Auckland District Health Board. Subject exclusion criteria were: history of ocular trauma or surgery, contact lens wear, ocular disease (other than keratoconus), corneal scarring and concurrent systemic disease or medication which may affect the cornea. Normal subjects with healthy corneas were recruited from staff and family and friends of patients attending the Ophthalmology clinic. Fully informed consent was obtained from each subject before examination. The study adhered to the tenets of the Declaration of Helsinki and had approval from the Northern X Regional Ethics Committee, Auckland.

The diagnosis of keratoconus was made based on a combination of clinical examination and corneal tomographic signs using the Pentacam rotating Scheimpflug tomographer (OCULUS Optikgerate GmbH, Wetzlar, Germany). The clinical diagnosis was made by a sub-specialist corneal surgeon based on clinical features of keratoconus including: corneal stromal thinning, Fleisher’s ring, Vogt’s striae and Munson’s sign. Tomographic features included abnormal
areas of central, inferior or superior steepening in conjunction with any of the following: oblique or non-orthogonal astigmatism $>1.5$D, maximum simulated keratometry greater than $47$D, or central corneal thickness less than $500\mu m$ as based on the Lim et al classification scheme for suspect keratoconics.  

Data were subsequently collected from the right eye of all subjects. Three tomographic parameters were analysed: 1) maximum simulated keratometry (Dioptres), 2) central corneal thickness (µm), and 3) posterior apical elevation above the computer generated posterior best fit sphere (µm).

ORA measurements were obtained with the subject in a sitting position. The subject was asked to fix on the central target of the non-contact probe. Measurements were obtained using automated alignment and release mechanisms initiated by the examiner. The examination was repeated until a measurement with a good signal score ($>5$) was obtained. The measurement with the best signal value for each eye was recorded as outlined in chapter 5 where the ORA produced repeatable measurements for readings with waveform scores of 5 or above. Corneal hysteresis (CH) and corneal resistance factor (CRF) were analysed.
6.3 **STATISTICAL ANALYSIS**

SPSS 15.0 for Windows data software (SPSS Inc Chicago, Illinois, USA) was used to perform Pearson correlation and general linear models comparing the correlations between the groups.

6.4 **RESULTS**

Forty-six subjects with keratoconus were recruited (31 males and 15 females) with a mean age of 24.7 ± 7.0 years (range 18 to 47 years). Sixty six healthy participants were recruited (25 males and 41 females) with a mean age of 27.4 ± 7.8 years (range 9 to 44 years). There was no significant difference in the mean ages of the two groups (P=0.10) (Table 6-1).

The mean maximum keratometry for the keratoconic group (53.4D ± 6.0D) was significantly higher than that for the normal group (43.9 ± 1.7D) (P<0.01) (Table 6-1). The mean apical posterior elevation for the keratoconic group (72.5 ± 49.2µm) was also significantly higher than that for the normal group (10.2 ± 31.4 µm) (P<0.01) (Table 6-1).

The mean corneal hysteresis (CH) for the keratoconic and normal groups was 8.3 ± 1.0 mmHg and 10.7 ± 1.8 mmHg respectively (P<0.01). The mean corneal resistance (CRF) for the keratoconic and normal groups was 6.7 ± 1.5 mmHg and 10.3 ± 1.9 mmHg respectively (P<0.01) (Table 6-1).

Posterior elevation was significantly and positively correlated with maximum keratometry in both the keratoconic (r = 0.69, p<0.01) and normal (r = 0.30, p<0.01) groups (Figure 6-1). There was no significant correlation between the posterior elevation and CH in the keratoconic group (r = -0.11, p= 0.49) and normal subjects (r = -0.12, p=0.36) (Figure 6-2).
Posterior elevation was significantly correlated with CRF in the keratoconic group ($r = -0.39$, $p< 0.01$). There was no significant correlation between these parameters in normal subjects ($r = -0.15$, $p=0.24$) (Figure 6-3).

![Graphic representation of the correlation between posterior elevation (µm) vs. Max K (D) in. Keratoconus ($r^2=0.474$) and normal corneas ($r^2=0.501$).](image)

**Figure 6-1**: Graphic representation of the correlation between posterior elevation (µm) vs. Max K (D) in. Keratoconus ($r^2=0.474$) and normal corneas ($r^2=0.501$).

Posterior corneal elevation correlated significantly with central corneal thickness in the keratoconic eyes ($r = -0.33$, $p=0.03$) and the normal group ($r = -0.37$, $p<0.01$). No significant difference was noted between the keratoconic and normal correlations ($p=0.46$) (Figure 6-4).

Central corneal thickness was significantly correlated with maximum keratometry in the keratoconic group ($r = -0.60$, $p<0.01$) but not in the normal group ($r = -0.2$, $p=0.47$). A significant difference was noted between the normal and keratoconic correlations with respect to both CCT and maximum keratometry ($p<0.01$).
Figure 6-2: Graphic representation of the correlation between posterior elevation (µm) vs. CH (mmHg) Keratoconus ($r^2=0.011$) and normal corneas ($r^2=0.013$).

Figure 6-3: Graphic representation of the correlation between posterior elevation (µm) and CRF (mmHg) in the Keratoconic ($r^2 = 0.012$) and normal groups ($r^2 = 0.233$).
Central corneal thickness was significantly correlated with CH in the normal group ($r=0.45, p<0.01$) but not in the keratoconic group ($r=0.25, p=0.10$). A significant difference was noted in CCT and CH between the normal and keratoconic corneas ($p=0.03$). Central corneal thickness was significantly correlated with CRF in both the keratoconic ($r=0.39, p=0.01$) and normal groups ($r=0.55, p<0.01$). A significant difference was also identified between the normal and keratoconic correlations between CCT and CRF ($p=0.04$).
### Table 6-1: Gender, age, topographic/tomographic parameters and ORA measures for the Keratoconus and normal control groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Keratoconus (N = 46)</th>
<th>Normal (N = 67)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender Ratio M:F</td>
<td>31:15</td>
<td>20:47</td>
</tr>
<tr>
<td>Age (years) (Mean ± std)</td>
<td>24.8 ± 7.0</td>
<td>25.2 ± 7.8</td>
</tr>
<tr>
<td>Age range (years)</td>
<td>18 - 47</td>
<td>9 - 43</td>
</tr>
<tr>
<td>Maximum Keratometry (D) (Mean ± std)</td>
<td>53.0 ± 6.0</td>
<td>43.9 ± 1.6</td>
</tr>
<tr>
<td>Maximum Keratometry (D) range</td>
<td>43.1 - 69.2</td>
<td>39.1 - 47.2</td>
</tr>
<tr>
<td>Central Corneal Thickness (µm) (Mean ± std)</td>
<td>470 ± 42</td>
<td>551 ± 31</td>
</tr>
<tr>
<td>Central Corneal Thickness (µm) range</td>
<td>352 - 553</td>
<td>471 - 617</td>
</tr>
<tr>
<td>Corneal Hysteresis (mmHg) (Mean ± std)</td>
<td>8.2 ± 1.1</td>
<td>10.6 ± 1.8</td>
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<tr>
<td>Corneal Hysteresis (mmHg) range</td>
<td>6.0 - 9.9</td>
<td>4.7 - 14.9</td>
</tr>
<tr>
<td>Corneal Resistance Factor (mmHg) (Mean ± std)</td>
<td>6.7 ± 1.5</td>
<td>10.3 ± 1.9</td>
</tr>
<tr>
<td>Corneal Resistance Factor (mmHg) range</td>
<td>4.0 - 10.2</td>
<td>5.5 - 14.8</td>
</tr>
<tr>
<td>Posterior apical elevation (µm) (Mean ± std)</td>
<td>132 ± 49</td>
<td>44 ± 20</td>
</tr>
<tr>
<td>Posterior apical elevation (µm) range</td>
<td>23 - 227</td>
<td>3 - 81</td>
</tr>
<tr>
<td>Posterior Best Fit Sphere (mm)(Mean ± std)</td>
<td>6.0 ± 0.45</td>
<td></td>
</tr>
<tr>
<td>Posterior Best Fit Sphere (mm) range</td>
<td>4.6 - 6.7</td>
<td></td>
</tr>
</tbody>
</table>
6.5 DISCUSSION

A large proportion of the intrinsic biomechanical properties of the cornea are the result of ordered stromal collagen fibrillar spacing and directionality. These properties have been shown to vary with age, corneal disease and secondary to ocular surgery. It has long been established that corneal biomechanical properties are affected in ectatic diseases such as keratoconus. The detection of ectatic disease is becoming increasingly important with the expanding field of corneal refractive surgery and the need to prevent iatrogenic disease in subjects prone to ectasia. The diagnosis of keratoconus is based on the integration of a number of clinical observations and measurements; with the early diagnosis of keratoconus often relying purely on examination of the corneal tomography/topography. The ORA was recently introduced as a non-invasive method of examining corneal biomechanical properties in vivo and as previously noted it has been suggested as a potential tool to aid in the diagnosis of keratoconus.

Whilst CH and CRF have been shown to be different between keratoconic and normal eyes there are limitations in using the ORA as a diagnostic tool. Shah et al noted that the difference in CH between mild and severe keratoconus was significant; however no significant difference was identified clinically between normal and mild, mild and moderate or moderate and severe disease. Significant overlap between the measures of the CH and CRF has also been described between patients with normal cornea and forme frusta, keratoconic suspect or mild keratoconics. Fontes et al. reported a large overlap and poor sensitivity when diagnosing keratoconus using a combination of the ORA parameters and Pentacam topography average keratometry, corneal astigmatism, corneal volume, anterior chamber depth and CCT.
The impact of CCT on the ORA measures cannot be ignored in screening for keratoconus. Kirwan et al.\textsuperscript{32} reported an overlap in CH and CRF values among patients with keratoconus and forme fruste keratoconus after eyes were matched for their corneal thickness.\textsuperscript{32}

The current study noted a strong correlation between posterior corneal elevation, the maximum keratometry and the central corneal thickness in both keratoconic and normal eyes. This is in agreement with previous studies that have noted strong relationships between corneal elevation\textsuperscript{33, 34} and corneal thickness\textsuperscript{33} in both keratoconus\textsuperscript{33} and normal\textsuperscript{34} populations.

This study established a significant correlation between CRF and posterior corneal elevation in the keratoconic but not in normal corneas. In contrast there was no significant relationship identified between CH and posterior elevation in either group. This suggests that CRF is potentially a more useful parameter than CH for identifying keratoconic biomechanical changes in the cornea. Conversely, Fontes et al.\textsuperscript{31} noted poor sensitivity and specificity (77.9% and 75.6% respectively) in differentiating keratoconus and healthy corneas based solely on CRF.\textsuperscript{31}

Corneal Hysteresis has been proposed as a measure of inherent viscous damping in the cornea whilst the CRF is thought to relate to the elastic resistance properties of the cornea giving a measure of its overall rigidity or resistance.\textsuperscript{21, 35} CRF has previously been reported to be more indicative of the mechanical strength of the cornea in keratoconus.\textsuperscript{36} This is rather than measuring the reduced rigidity that is largely related to decreased central corneal thickness alone.\textsuperscript{37}

The current study established significant relationships between CCT and both CH and CRF in the normal and in the keratoconic eye. These results agree with the data of Shah et al.\textsuperscript{21} that highlighted moderate correlations between CCT and both CH and CRF. This suggests that
CCT is a significant modifier for ORA measures and should be accounted for in diseases where CCT varies significantly from normal values e.g. keratoconus where the central corneal thickness may be markedly reduced. However, the manufacturers do not directly incorporate an individual’s CCT as a factor in calculations, and it is simply incorporated as a generic constant. This is based upon the manufacturer’s contention that area density (mass/unit area) does not significantly contribute to differences observed between the ORA inward and outward signal peaks. This is illustrated by the fact that eyes with Fuchs’ endothelial dystrophy have higher corneal volume (due to increased corneal thickness) yet demonstrate reduced CH compared to corneas in normal eyes.

The current study identified significant correlations between posterior corneal elevation and both maximum keratometry and CCT in keratoconic and normal eyes. It also noted a significant correlation between posterior elevation and CRF solely in the keratoconic group. As many studies have already shown the CH and CRF indices alone are poor discriminators of keratoconic from normal corneas. Based on the results of the current study the author suggests that combining posterior elevation, maximum keratometry and central corneal thickness should increase the diagnostic sensitivity whilst also providing a better insight into the ectatic disease process. To investigate this further a large group of keratoconic eyes with a wide range of severity would be required and thus presents opportunity for further research into the efficacy of the ORA as an adjunctive diagnostic tool for keratoconus.
REFERENCES


SECTION IV CORNEAL COLLAGEN CROSS-LINKING

(CHAPETERS 7-9)
Chapter 7 : Methods
7.1 INTRODUCTION:

Corneal collagen cross-linking has recently been introduced as a method of treating keratoconus. It involves inducing cross-links between collagen fibrils in the cornea aiming to increase the mechanical rigidity and thereby enabling the tissue to resist further protrusion. This treatment method is only indicated in early to moderate keratoconus aiming to halt or slow down the progression of the disease at a stage when the vision can still be maximized with spectacles or contact lenses.

The aim of this study was to investigate the efficacy of corneal collagen cross-linking in progressive keratoconus. The study hypothesis being: progression of keratoconus ectasia in collagen cross-linking treated eyes is halted or greatly reduced, whereas, untreated control eyes continue to progress.

7.2 PARTICIPANTS

The current study was designed as a prospective randomised controlled trial of corneal collagen cross-linking in subjects with progressive keratoconus. This study was conducted according to the principles of the Declaration of Helsinki. This study received approval from the Northern X Ethics committee (NTX/08/08/070). Informed written consent was obtained from all patients prior to enrolment. Patients were recruited from ophthalmology and optometry practices across New Zealand. Inclusion and exclusion criteria were used based on published international studies on safety and efficacy of corneal collagen cross-linking.
7.2.1 Study Group: Inclusion Criteria

- Bilateral keratoconus
- Documented progressive keratoconus
- Age >14 years and <30 years
- Maximal corneal keratometry of ≤60 D
- Minimal corneal thickness of at least 400 µm
- Best spectacle corrected visual acuity of ≥20/80 (6/24)
- Clear cornea on slit-lamp biomicroscopy with no scarring or Vogt's striae
- Informed patients with the ability to understand the implications of the intervention, such that they were able to provide fully informed consent for treatment
- Documented rigid gas permeable contact lens intolerance

7.2.2 Study Group: Exclusion Criteria

- Previous episodes of corneal hydrops
- Prior ocular surgery or trauma
- Systemic disease which may affect the cornea
- Demonstrated corneal topographical stability in the preceding 24 months
- Previous herpetic disease of the cornea
- Significant corneal desiccation staining (Grade 2 on the Cornea and Contact Lens Research Unit (CCLRU) grading scale).  
- Minimal corneal thickness of less than 400 µm
- Corneal stromal scarring or Vogt's striae on slit-lamp biomicroscopy
- Inability to give informed consent
- Inability to attend follow up appointments over a period of months
7.2.3 Definition of Progression

- At least 6 months of preceding refractive/ topographic/ keratometric data available to accurately assess the rate of progression
- Progression of disease was defined as at least one of the following:
  - Increase in maximal keratometry of ≥0.75D within the preceding three months
  - Change in refractive astigmatism of ≥0.75D within the preceding 12 months
  - Progression measured indirectly by using rigid contact lenses of varying base curves to achieve apical clearance. A change of ≥0.2mm in base curve in documented contact lens fit in the last 6-12 months was considered significant.
  - Demonstrated decrease in corneal thickness of ≥80µm in preceding 6 months.

7.2.4 Randomization

A list containing participant identification numbers and the eye to be treated (right or left) was randomly generated prior to the start of the study. Participant identification numbers were issued consecutively to subjects as they were recruited. Once a study number was allocated, the treatment eye was determined by consulting the previously generated list. The untreated eye acted as the control for the study.

7.3 CROSS-LINKING PROCEDURE

The treatment protocols used in this study were adapted from those reported by Wollensak et al. Procedures were performed under aseptic conditions in a dedicated procedure room. Pre-operative central corneal thickness was measured using ultrasound pachymetry. The UVA light source used was the SDZ X-Linker (ADX Electronics Ltd, Christchurch, New Zealand) (Figure 7-1). The UVA light source was calibrated before each procedure. The
calibrator was placed under the light source and moved backwards and forwards to obtain a maximum reading. The maximum reading was documented as being no greater than 2.7mW/cm². This was equivalent to 3.0mW for a parallel beam light source.

The patient was asked to lie in a supine position. Their head was placed within a rest to maintain stability during the procedure. Preservative free tetracaine hydrochloride 1% eye drops (Chauvin Pharmaceuticals, Surrey, UK) were instilled in the treatment eye. The external eye and adnexa was then cleaned with Chlorhexidine solution (0.02%, Baxter Healthcare Pty. Ltd, Toongabbie, N.S.W. Australia) and allowed to dry. A sterile drape was placed over the eye and a sterile lid speculum inserted.

![A. SDZ X-Linker colour monitor with 30 min countdown timer ensuring accurate positioning and timing. B. The UVA light source with adjustable head.](image)

**Figure 7-1:** A. The SDZ X-Linker colour monitor with 30 min countdown timer ensuring accurate positioning and timing. B. The UVA light source with adjustable head.

A corneal shield soaked in preservative free tetracaine hydrochloride 1% was placed on the cornea for 30 seconds to loosen the corneal epithelium prior to debridement. The cornea was marked using a 7 or 8mm gun sight corneal marker. The area to be debrided was dependant on the patient's corneal diameter and location of the cone.
The central 7-8 mm of the corneal epithelium was mechanically removed using a bevel-up crescent blade (Figure 7-1). Ultrasound pachymetry was then performed to ascertain the type of riboflavin to instill. For corneas with a post-epithelial debridement corneal thickness of ≥400 µm, 0.1% solution of isotonic riboflavin ophthalmic solution was instilled (10mg riboflavin-5-phosphate in 10mls dextran-T-500 solution, Opto Ribolink, Opto Global Pty. Ltd, Australia). For corneas with a post epithelial debridement corneal thickness of less than 400 µm, hypotonic riboflavin 0.1% ophthalmic solution (Opto Ribolink, Opto Global Pty. Ltd, Australia) was instilled.

Following epithelial debridement riboflavin drops were instilled every 5 minutes for 30 minutes. Central corneal pachymetry was performed every 5 minutes during the riboflavin instillation, to ensure that the corneal thickness remained above 400 µm at all times. If the corneal thickness fell below 400 µm hypotonic riboflavin solution was instilled until the thickness returned to, or was greater than, the recommended level for safety. The procedure was modified slightly after the first 3 patients because significant corneal thinning was noted during each procedure. A corneal shield sponge soaked in riboflavin solution was applied to the debrided area. Riboflavin drops were then applied to the shield every 5 minutes. Central corneal thickness measurements were performed every 5 minutes, after temporarily removing the shield.

Confirmation of riboflavin penetration was performed by assessing for yellow flare in the anterior chamber; this was carried out using the white light of the surgical microscope. Light irradiation of the cornea was then commenced using a UVA double diode 370 nm light source located 10-12mm in front of the corneal apex. This produced a radiant energy of 3mW/cm² or 5.4 J/cm² (monitored via a potentiometer/UV power meter) (Figure 7-2). Irradiation was performed for 30 minutes with further instillation of riboflavin drops every 5 minutes. Pachymetry measurements were performed every 5 minutes throughout the procedure.
Upon completion of the procedure the eye was flushed with balanced salt solution and a bandage contact lens (Base Curve 8.6, Diameter 14mm Bioinfinity from CooperVision, Fairport, NY) was inserted. Preservative free chloramphenicol eye drops 0.5% (Chauvin Pharmaceuticals, Surrey, UK) were instilled and dispensed to the patients.

Figure 7-2: A. The UVA lamp aligned 10-12mm in front of the corneal apex to obtain radiant energy of 3mW/cm² or 5.4 J/cm² following riboflavin installation. B. The subject is in the supine position with the UVA lamp being aligned from above. C. An 8mm central corneal debridement area with riboflavin drops instilled imaged by slit lamp biomicroscopy using a cobalt blue light filter.
A detailed instruction sheet was given to each patient regarding topical antibiotic use and a single minim of preservative free oxybuprocaine hydrochloride 0.4% (Chauvin Pharmaceuticals, Surrey, UK) was provided as emergency drops for severe pain in the first 24 hours. Oral Panadeine (Paracetamol 500mg and Codeine phosphate 15mg) 2 tablets every 4-6 hours was prescribed for pain relief for the first ten patients. However, nine of the first ten patients reported unsatisfactory pain relief and related insomnia for the first two days. Following this the analgesic regimen was subsequently altered to a combination of oral Tramadol (50mg every 4-6 hours) and oral Diazepam (4mg nocte) for the first four days post-operatively.

Follow up examinations were scheduled for day 1, day 3, 1 week, 1 month, 3 months, 6 months, 12 months and 24 months post-operatively.

7.4 EXAMINATION PROTOCOL

A comprehensive ocular and medical history was obtained; this included contact lens history, history of atopy or eye rubbing and family history of keratoconus.

7.4.1 Visual Acuity

Visual acuity was assessed using a Snellen visual acuity chart at a distance of 6 meters. Unaided, aided (with spectacles or contact lenses) and pinhole visual acuity were assessed. Snellen visual acuity was then converted to LogMAR for the purpose of statistical analysis. Refraction was performed by a single experienced examiner (CJ) on both the treated and control eyes at the 3, 6, 12 and 24 month follow up reviews.
7.4.2 Slit Lamp Examination

Slit lamp biomicroscopy and corneal photography was performed pre-operatively and at all post-operative examinations.

7.4.3 Corneal Tomography

Pentacam rotating Scheimpflug tomography (Oculus, Wetzlar, Germany) was performed on both eyes of each patient pre-operatively and at all post-operative examinations. The tomographic parameters analysed at each time point included:

1. Simulated keratometry (Max and Min K)
2. Simulated corneal astigmatism and axis
3. Central corneal thickness
4. Thinnest point corneal pachymetry
5. Anterior best fit sphere (BFS) and anterior elevation above the BFS
6. Posterior BFS and posterior elevation above the BFS.
7. Computer generated corneal volume from the central 7mm

Scans were excluded if the scan quality was not adequate. This was determined by the Pentacam inbuilt software which indicates scan quality. Scans were excluded if any error message was present following the scan.

To obtain elevation values the computer generated best fit sphere was utilized. The greatest elevation values were manually acquired by placing the mouse over the highest point of the best fit sphere and reading off the associated scale value. The mean keratometry was manually extrapolated from the computer generated simulated keratometry values.
7.4.4 Higher Order Aberration Wavefront Analysis

The Oculus Pentacam Zernike analysis was used to measure first surface corneal aberrations for both eyes of each patient pre-operatively and at all post-operative examinations.

7.4.5 Densitometry

As an objective measure of collagen cross-linking associated corneal haze, corneal densitometry was measured along one meridian (steepest meridian (MaxK) measured at the pre-operative examination) using the Scheimpflug image. The Pentacam quantifies the density of the cornea on a scale from 0 to 100.

7.4.6 Corneal Biomechanics

Corneal hysteresis (CH) and corneal resistance factor (CRF) were assessed using the Ocular Response Analyzer (ORA; Reicherts Ophthalmic Instruments Inc., Depew, New York, USA) as described in Chapter 2. Examinations were performed on both eyes of each patient pre-operatively and at all post-operative examinations. A measurement with a quality score above 5 was considered accurate, however, if this was not possible three consecutive measurements were averaged.

7.4.7 In-Vivo Confocal Microscopy

Laser scanning in vivo confocal microscopy (IVCM) was performed on all subjects using the Heidelberg Retina Tomograph II, Rostock Corneal Module [RCM] (Heidelberg Engineering GmbH, Heidelberg, Germany).
The treatment eye was anesthetized with a drop of minims oxybuprocaine hydrochloride 0.4% (Chauvin Pharmaceuticals, Romford, UK). Viscotears gel (Carbomer 980, 0.2%; Novartis, Basel, Switzerland) was used as a coupling agent between the applanating lens cap and the microscope lens. During the examination, all subjects were asked to fix on a distant target so that the central cornea was examined.

The cornea was scanned using the device’s ‘section mode’ enabling instantaneous imaging of a single area of the cornea at a desired depth. The total duration of IVCM examination was approximately 2 minutes per eye, and no subjects experienced any visual or corneal complications following the examination. For each IVCM examination two images were selected from each of the following layers: basal epithelium, sub basal nerve plexus, anterior stroma, and posterior stroma. The selection process is outlined in section 7.4.8.

IVCM images of the corneal endothelium were obtained using the Confoscan 4 (Nidek Technologies, Gamagori, Japan). This system was chosen for endothelial imaging and analysis because good intra-observer and inter-observer reproducibility of endothelial density measurement using the automatic endothelial analysis has been previously reported. In contrast corneal endothelial cell density has been shown to be significantly overestimated with the RCM laser scanning system.

7.4.8 Image Analysis

For each time point two of the clearest images from each layer were selected by an experienced observer (CJ). Images of the basal epithelium were selected as being the first clear image immediately anterior to Bowman’s layer. The sub basal nerve plexus images were defined as the first clear images of the nerves at the level of Bowman’s layer. The anterior stromal images were defined as the first clear images immediately posterior to Bowman’s layer whilst the posterior stromal images were defined as the first clear images
immediately anterior to the endothelium. All selected images were randomized by an independent examiner (NA). Quantitative analysis was subsequently performed by a single examiner (CJ) using Image J software (U. S. National Institutes of Health, Bethesda, Maryland, USA) for keratocyte density and analySIS software (analySIS 3.1Soft Imaging System, Münster, Germany) for sub-basal nerve density.

The full 400 x 400µm frame of each sub-basal nerve plexus and stromal image was used for analysis. Only keratocyte nuclei that were in focus, with all edges within the image frame were analysed and counted. Sub-basal nerve density was calculated by measuring the total length of the nerves per image.

### 7.4.9 Statistical Analysis

All data and statistical analyses were considered in conjunction with a senior biostatistician before the commencement of the study and following data acquisition.

The statistical programme SAS (Statistical Analysis System Version 9.1, SAS Institute Inc. Cary, NY, USA) was utilised to perform univariate and multivariate analysis.

Repeated measures ANOVA was performed for all outcome parameters. Differences from pre-operative measures were analysed to exclude the influence of individual subjects not attending all post-operative appointments.

Student t-Test was used to examine differences between the treatment and control groups at different post-operative time points.

Each outcome variable was analysed using general linear mixed models accounting for random coefficients. Tests of fixed effects were used to assess the impact of several factors;
Chapter 7

namely the presence of a family history of keratoconus, atopy (eczema, asthma and allergies), self-reported eye rubbing, and disease severity (MaxK). Statistical significance was defined as p<0.05.

Ten percent of all images were re-analysed by the same and by an independent examiner (Nandoun Abeysekera) to determine intra-examiner and inter-examiner repeatability.
REFERENCES


5. SDZ Electronics. http://www.sdz.co.nz


Chapter 8: Results
8.1 INTRODUCTION

A total of 39 patients (28 male and 11 female) were recruited for this study. The mean age of patients was 21.9 ± 6.0 years. Twenty-two right eyes and 17 left eyes were treated. One patient was excluded from the study after developing microbial keratitis within the first post-operative week (see clinical outcomes) and one further patient was lost to follow up one month post-treatment. Thirty-three (86.8%) patients returned for one month follow-up, 31 (81.6%) returned at three months, 33 returned at six months (86.8%), 22 (57.9%) patients returned at twelve months and only 6 (15.8%) had reached the twenty four months assessment. No patients elected to withdraw from the study. Of the treated patients that showed continued progression in the fellow eye 13 (33%) chose to have the fellow eye treated at least six months after the initial treatment.

8.2 CLINICAL OUTCOMES

Of the 39 patients treated with collagen cross-linking five patients required the use of hypotonic riboflavin solution because the corneal thickness had fallen below 400µm during the treatment episode.

The corneal epithelial defect had fully re-epithelialised by day four post-operatively in 32 patients (82%) and the remaining 6 patients (13.2%) the cornea had completely re-epithelialised by day 7 post-operatively (Figure 8-1).

All patients reported cloudy vision and light sensitivity at day three which persisted for up to one month post-operatively. At the one month post-operative assessment anterior stromal haze with a stromal demarcation line was noted in 25 of the 33 (76%) patients examined.
Figure 8-1: A. Pseudo-dendrite appearance indicating fusion of the healing edges of the corneal epithelium (four days post-operatively). B. Superior edge of zone of corneal stromal haze indicating transition between treated and non-treated cornea (imaged at one month post-operatively). C D Optical section of anterior stromal corneal haze (imaged one month post-operatively) (25x and 40x magnification respectively) E. Optical section of the demarcation line of the anterior stromal corneal haze (imaged three months post-operatively x25 magnification)

In three patients ocular discomfort associated with light sensitivity persisted beyond the one month post-operative examination. This required a short tapered course of gutte.Predforte 1% (prednisolone acetate ophthalmic suspension 1.0%, Allergan, Irvine, CA, USA) instilled four times a day for 3-4 weeks to reduce the patients’ discomfort. At three months one patient continued to report mild hazy vision.
Figure 8-2: A. Mid-peripheral sterile infiltrate observed at day five post-operatively in an otherwise uninflamed eye; B. Mild anterior uveitis with cross-linking associated corneal haze and marked circumciliary injection at three weeks post-operatively; C. Severe bacterial keratitis presenting three days post-operatively; D. Scar formation following resolution of bacterial keratitis (patient in image C) six months post-operatively.

One patient contacted the principal investigator due to increasing symptoms of light sensitivity three weeks post-operatively and was noted to have a mild anterior uveitis with circumciliary injection and significant corneal haze (Figure 8-2). The patient was treated with gutte. Predforte 1% instilled four times a day and tapered to nil over one month. This was associated with elimination of the patient’s symptoms and resolution of the clinical signs. In two patients sterile corneal infiltrates were observed at day 5 and 1 week respectively (Figure 8-2). These patients were treated with gutte. Ciloxan four times a day (ciprofloxacin hydrochloride 0.3%) (Alcon, Fort Worth, Texas, USA) for one week followed
by gutte.Predforte 1% four times a day, tapered to nil treatment over a subsequent two
week period.

Figure 8-3: A. Deep stromal haze in the central cornea at six months post-operatively; B. Pre-
Descemet’s membrane haze observed three months post-operatively.

One patient presented on day 3 post-operatively with a painful red eye, and slit lamp
examination revealed a dense central corneal infiltrate suggestive of microbial keratitis
(Figure 8-2). Culture of a corneal scrape specimen grew Staphylococcus aureus. The
patient was treated with fortified topical antibiotics (gutte.tobramycin 1.3% and
gutte.cefuroxime 5%) hourly day and night for 2 days and then slowly tapered to six times a
day over 1 week. On discharge from hospital the patient was prescribed gutt Ciloxan six
times a day. The infection fully resolved over the following three weeks. A stromal scar was
noted following resolution of the infection (Figure 8-2). Despite this significant complication,
corneal flattening was associated with improved unaided visual acuity from 0.9 LogMAR
(6/48) pre-operatively to 0.48 LogMAR (6/18) post-operatively.
Five patients (12.8%) developed relatively dense central posterior stromal haze (Figure 8-3). In two of these patients the stromal haze was noted at the one month post-operative assessment. In the remaining three patients the haze was noted three months post-operatively. The corneal haze was not associated with any change in endothelial density when compared to pre-operative measurements (p=0.7). The haze had completely resolved by twelve months post-operatively without intervention.

In two patients a mid-stromal ground glass appearance was identified on retro-illumination three months post-operatively (Figure 8-4), however, both patients were asymptomatic and the appearance was persistent but diminished by 12 and 24 months post-operatively.

### 8.3 VISUAL ACUITY AND REFRACTION

#### 8.3.1 Uncorrected Visual Acuity (UCVA)

The mean uncorrected visual acuities for both treated and control eyes at each time point, before and after surgery, are detailed in Table 8.1.
Compared to pre-operative measurements the mean UCVA in treated eyes improved significantly at three (-0.19 ± .28 LogMAR, p<0.01), six (-0.15 ± 0.29 LogMAR, p<0.01) and at twelve months (-0.22 ± 0.26 LogMAR, p<0.01) following the collagen cross-linking procedure (Table 8-1).

<table>
<thead>
<tr>
<th>Review time point</th>
<th>Number of subjects</th>
<th>Mean UCVA ± std LogMAR (Snellen equivalent)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treated eyes</td>
<td>Control untreated eyes</td>
</tr>
<tr>
<td>Pre-operative</td>
<td>38</td>
<td>0.77 ± 0.35 (6/35)</td>
<td>0.63 ± 0.43 (6/26)</td>
</tr>
<tr>
<td>1 month post-operative</td>
<td>33</td>
<td>0.67 ± 0.36 (6/28)</td>
<td>0.58 ± 0.42 (6/23)</td>
</tr>
<tr>
<td>3 months post-operative</td>
<td>31</td>
<td>0.58 ± 0.35 (6/23)</td>
<td>0.59 ± 0.43 (6/23)</td>
</tr>
<tr>
<td>6 months post-operative</td>
<td>33</td>
<td>0.55 ± 0.38 (6/21)</td>
<td>0.54 ± 0.45 (6/21)</td>
</tr>
<tr>
<td>12 months post-operative</td>
<td>22</td>
<td>0.57 ± 0.36 (6/22)</td>
<td>0.50 ± 0.36 (6/19)</td>
</tr>
<tr>
<td>24 months post-operative</td>
<td>6</td>
<td>0.51 ± 0.22 (6/19)</td>
<td>0.14 ± 0.24 (6/8)</td>
</tr>
</tbody>
</table>

Table 8-1: Mean uncorrected visual acuity (UCVA) in the treated and control eyes. (LogMAR Snellen equivalent).

However, although a significant difference in UCVA was identified in treated eyes compared to pre-operative values at several time points, a significant difference in UCVA between the treated and control eyes was only noted at 24 months post operatively (Table 8-2).
<table>
<thead>
<tr>
<th>Review Time Point</th>
<th>Number of Subjects</th>
<th>Mean change in UCVA ± std (D) compared to pre-op</th>
<th>p value (Control vs. Treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treated eyes</td>
<td>Control untreated eyes</td>
</tr>
<tr>
<td>1 month</td>
<td>33</td>
<td>-0.08 ± 0.15 p=0.09</td>
<td>-0.09 ± 0.15 p=0.09</td>
</tr>
<tr>
<td>3 months</td>
<td>31</td>
<td>-0.19 ± 0.28 p&lt;0.01</td>
<td>-0.12 ± 0.22 p=0.90</td>
</tr>
<tr>
<td>6 months</td>
<td>33</td>
<td>-0.15 ± 0.29 p&lt;0.01</td>
<td>-0.06 ± 0.21 p=0.10</td>
</tr>
<tr>
<td>12 months</td>
<td>22</td>
<td>-0.22 ± 0.26 p&lt;0.01</td>
<td>0.01 ± 0.16 p=0.90</td>
</tr>
<tr>
<td>24 months</td>
<td>6</td>
<td>-0.26 ± 0.30 p=0.05</td>
<td>-0.05 ± 0.1 p=0.31</td>
</tr>
</tbody>
</table>

**Table 8-2:** Change in uncorrected visual acuity (UCVA) in treated and control eye between one and 24 months after collagen cross linking. At each time point in column 3 and column 4 the p-value expressed relates to the UCVA at that time point compared to baseline pre-operative UCVA. Column 5 highlights UCVA for control compared to treated eyes at each time point.
Figure 8-5: Mean Uncorrected Visual Acuity (UCVA) (LogMAR) in control and treated eyes preoperatively and at 1, 3, 6 and 12 month follow up appointments.

8.3.2 Best Corrected Visual Acuity (BCVA)

The mean BCVA for treated and control eyes for each time point are detailed in Table 8-3 and Figure 8-6. Compared to pre-operative measurements the mean BCVA in treated eyes only improved significantly at 3 months post-operatively (-0.08 ± 0.15, p=0.01) (Table 8-4).

There was no significant variation in BCVA at any time point when compared to baseline in control eyes. There was no significant difference in BCVA between the treated and control eyes at any examined time point (Table 8-4, Figure 8-6).

When analysing BCVA data using general linear mixed models accounting for random coefficient there was a trend to improved best corrected visual acuity over the follow up
period. This trend was greatest in patients with a family history of keratoconus (p=0.08) and the presence of asthma (p=0.08). It should be noted that subjects with a history of asthma had improved BSCVA post-operatively compared to those without; this however did not reach statistical significance. No interactions were noted over time between any of the factors identified.

<table>
<thead>
<tr>
<th>Review time point</th>
<th>Number of Subjects</th>
<th>Mean BSCVA ± std LogMAR (Snellen equivalent)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treated eyes</td>
<td>Control untreated eyes</td>
</tr>
<tr>
<td>Pre-operative</td>
<td>38</td>
<td>0.26 ± 0.17 (6/12)</td>
<td>0.20 ± 0.17 (6/9)</td>
</tr>
<tr>
<td>1 month post-operative</td>
<td>31</td>
<td>0.28 ± 0.18 (6/12)</td>
<td>0.20 ± 0.21 (6/9)</td>
</tr>
<tr>
<td>3 months post-operative</td>
<td>31</td>
<td>0.20 ± 0.13 (6/9)</td>
<td>0.21 ± 0.19 (6/9)</td>
</tr>
<tr>
<td>6 months post-operative</td>
<td>33</td>
<td>0.23 ± 0.16 (6/10)</td>
<td>0.18 ± 0.21 (6/9)</td>
</tr>
<tr>
<td>12 months post-operative</td>
<td>22</td>
<td>0.20 ± 0.13 (6/9)</td>
<td>0.16 ± 0.13 (6/9)</td>
</tr>
<tr>
<td>24 months post-operative</td>
<td>6</td>
<td>0.16 ± 0.09 (6/9)</td>
<td>0.18 ± 0.18 (6/9)</td>
</tr>
</tbody>
</table>

**Table 8.3**: Mean Best Corrected Visual Acuity (BCVA) (LogMAR (Snellen Equivalent)) in treated and control eyes pre-operatively and at standardised time-points up to 24 months.
<table>
<thead>
<tr>
<th>Review time point</th>
<th>Number of Subjects</th>
<th>Mean change in BCVA ± std (LogMAR) compared to pre-op</th>
<th>p value (Control vs. Treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treated eyes</td>
<td>Control Untreated eyes</td>
</tr>
<tr>
<td>1 month post-operative</td>
<td>33</td>
<td>0.02 ± 0.21</td>
<td>-0.01 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.76</td>
<td>p=0.44</td>
</tr>
<tr>
<td>3 months post-operative</td>
<td>31</td>
<td>-0.08 ± 0.15</td>
<td>-0.03 ± 0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.01</td>
<td>p=0.80</td>
</tr>
<tr>
<td>6 months post-operative</td>
<td>33</td>
<td>-0.03 ± 0.12</td>
<td>-0.08 ± 0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.21</td>
<td>p=0.52</td>
</tr>
<tr>
<td>12 months post-operative</td>
<td>22</td>
<td>-0.07 ± 0.19</td>
<td>-0.04 ± 0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.38</td>
<td>p=0.52</td>
</tr>
<tr>
<td>24 months post-operative</td>
<td>6</td>
<td>-0.13 ± 0.01</td>
<td>-0.04 ± 0.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.41</td>
<td>p=0.42</td>
</tr>
</tbody>
</table>

Table 8-4: Change in Best Corrected visual acuity (BCVA) in treated and control eyes (pre-operative to 24 months post-operatively) at each time point in column 2 and column 3 the p-value expressed relates to the BCVA at that time point compared to baseline pre-operative BCVA. Column 4 highlights statistical analysis of BCVA for treated eyes compared to control at each time point.
**Figure 8-6**: Mean Best Corrected Visual Acuity (BCVA) (LogMAR) in treated and control eyes preoperatively and at 1, 3, 6 and 12 month following collagen cross-linking.

### 8.3.3 Refractive Spherical Equivalent

The mean refractive spherical equivalent for treated and control eyes for each time point is detailed in Table 8-5. There was no significant change in the mean refractive spherical equivalent at any time point when compared to baseline in treated and control eyes (Table 8-5).

There was also no significant difference in the mean refractive spherical equivalent between treated and control eyes at any time point. When analysing refractive spherical
equivalent using general linear mixed models accounting for random coefficient no
significant interactions were noted over time between any of the factors identified.

<table>
<thead>
<tr>
<th>Review time point</th>
<th>Number of Subjects</th>
<th>Mean refractive Spherical Equivalent ± std (D) compared to pre-op</th>
<th>p value (Control vs. Treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treated eyes</td>
<td>Control Untreated eyes</td>
</tr>
<tr>
<td>Pre-operative</td>
<td>38</td>
<td>-2.66 ± 3.9 D</td>
<td>-1.95 ± 3.8 D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.78</td>
<td></td>
</tr>
<tr>
<td>1 month post-operative</td>
<td>33</td>
<td>-2.67 ± 4.8 D</td>
<td>-2.30 ± 2.1 D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.78</td>
<td></td>
</tr>
<tr>
<td>3 months post-operative</td>
<td>31</td>
<td>-2.63 ± 4.3D</td>
<td>-3.00 ± 4.1 D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.99</td>
<td></td>
</tr>
<tr>
<td>6 months post-operative</td>
<td>33</td>
<td>-2.08 ± 4.3D</td>
<td>-2.14 ± 3.5 D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.27</td>
<td></td>
</tr>
<tr>
<td>12 months post-operative</td>
<td>22</td>
<td>-1.89 ± 3.5D</td>
<td>-2.50 ± 3.6D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.33</td>
<td></td>
</tr>
</tbody>
</table>

Table 8-5: Refractive Spherical Equivalent (D) in treated and control eyes at pre-operative and 1, 3, 6 and 12 month reviews following collagen cross-linking. Statistics in columns two and three compare data to pre-operative values. Statistical values in column four compare the mean change in refractive spherical equivalent between treated and control (untreated) eyes at each time point.
8.4 CORNEAL TOMOGRAPHY/ TOPOGRAPHY

8.4.1 Maximum Keratometry

Maximum keratometry values for each review time point are detailed in Table 8-6 and Figure 8-7. Compared to pre-operative values a significant increase in the mean maximum keratometry was noted in the treated eyes at one month post-operatively (0.64 ± 1.3 D, p=0.01). Significant decreases in the mean maximum keratometry were observed at six months (-0.6 ± 1.2 D, p<0.01), twelve months (-1.20 ± 2.0 D, p=0.02) and 24 months post-operatively (-1.48 ± 0.67 D, p=0.01) (Table 8-7).

At one month post-operatively 22 of the 31 (66.7%) treated eyes examined showed steepening of maximum keratometry by a mean of 1.23 ± 1.23 D. At six months post-operatively 4 of the 33 (12%) treated eyes had progressed (≥0.75D) by a mean of 2.4 ± 1.49 D. At twelve months 2 of the 22 (9.1%) treated eyes showed a mean progression of 1.0 ± 0.28 D.

Compared to baseline values there were significant increases in maximum keratometry in the control eyes at three (0.50 ± 0.94 D, p<0.01), six (0.61 ± 1.40D, p=0.01), and twelve months (0.72 ± 1.41D, p=0.03) (Table 8-7). At six months 14 of the 33 eyes (42.4%) in the control group had progressed (0.75D) by a mean of 1.82 ±1.33 D. At twelve months 7 of the 22 (31.8%) control eyes examined had progressed (0.75D) by a mean of 2.3 ± 1.2D.

A significant difference was noted in keratometry between the treated and control eyes pre-operatively (p=0.03) and at 1 month (p<0.01) with the treated eyes having steeper mean maximum keratometry. However, at 3, 6, 12, and 24 months post-operatively there was no significant difference between the groups (Table 8-7). When analysing the maximum
keratometry data using general linear mixed models accounting for random coefficient there was a significant difference in the change in maximum keratometry over the follow up period between the treated and control groups (p=0.03), with the treatment group showing significant regression.

<table>
<thead>
<tr>
<th>Review time point</th>
<th>Number of Subjects</th>
<th>Mean maximum Keratometry ± std (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treated eyes</td>
</tr>
<tr>
<td>Pre-operative</td>
<td>38</td>
<td>53.2 ± 4.9</td>
</tr>
<tr>
<td>1 month post-operative</td>
<td>31</td>
<td>53.7 ± 4.6</td>
</tr>
<tr>
<td>3 months post-operative</td>
<td>31</td>
<td>53.1 ± 5.18</td>
</tr>
<tr>
<td>6 months post-operative</td>
<td>33</td>
<td>53.2 ± 5.4</td>
</tr>
<tr>
<td>12 months post-operative</td>
<td>22</td>
<td>52.3 ± 4.9</td>
</tr>
<tr>
<td>24 months post-operative</td>
<td>6</td>
<td>53.8 ± 4.8</td>
</tr>
</tbody>
</table>

Table 8-6: Mean maximum keratometry (D) in treated and control eyes at pre-operative and 1, 3, 6 and 12 month reviews following collagen cross-linking. A decrease in mean maximum keratometry is noted over the review period in the treated eyes; however statistical analysis of this data did not reach significance.
There were no significant interactions between the treated eyes and control group outcomes when analysing for the presence of; atopy (eczema (p=0.34), asthma (p=0.95) and allergies (p=0.96)), family history (p=0.83), eye rubbing (p=0.19) and severity of disease (p=0.70).

<table>
<thead>
<tr>
<th>Review time point</th>
<th>Number of Subjects</th>
<th>Mean change in Max K ± std (D) compared to pre-op</th>
<th>p value (Control untreated eyes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treated eyes</td>
<td>Control untreated eyes</td>
</tr>
<tr>
<td>1 month post-operative</td>
<td>33</td>
<td>0.64 ± 1.30</td>
<td>0.23 ± 0.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.01</td>
<td></td>
</tr>
<tr>
<td>3 months post-operative</td>
<td>31</td>
<td>-0.18 ± 1.10</td>
<td>0.50 ± 0.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.36</td>
<td></td>
</tr>
<tr>
<td>6 months post-operative</td>
<td>33</td>
<td>-0.60 ± 1.20</td>
<td>0.61 ± 1.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>12 months post-operative</td>
<td>22</td>
<td>-1.20 ± 2.0</td>
<td>0.72 ± 1.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.02</td>
<td></td>
</tr>
<tr>
<td>24 months post-operative</td>
<td>6</td>
<td>-1.48 ± 0.67</td>
<td>0.73 ± 0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

**Table 8-7:** Change in maximum keratometry (D) in treated and control eyes at 1, 3, 6, 12 and 24 month reviews following collagen cross-linking. Statistics in columns two and three compare data to pre-operative values. Statistical values in column four compare the mean change in maximum keratometry between treated and control (untreated) eyes at each time point.
8.4.2 Average Keratometry

Mean average keratometry values for each time point is detailed in Table 8-8 and Figure 8-8. Compared to the pre-operative values a significant increase in the average maximum keratometry was noted in the treated eyes at one month post-operatively (0.41 ± 1.00D, p=0.03) (Table 8-9). At 1 month post-operatively 12 of 31 (38.7%) treated eyes exhibited steepening of average keratometry (≥0.75D) by a mean of 1.35 ± 0.64 D when compared to pre-operative values. Compared to the pre-operative measurements significant decreases in the mean average keratometry were observed in the treated eyes at three (-0.40 ± 0.81 D, p=0.01), six (-0.56 ± 1.06 D, p<0.01), and 12 months post-operatively (-0.68 ± 1.18 D, p=0.01) (Table 8-9).

<table>
<thead>
<tr>
<th>Review time point</th>
<th>Number of Subjects</th>
<th>Mean Average Keratometry ± std (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treated eyes</td>
</tr>
<tr>
<td>Pre-operative</td>
<td>38</td>
<td>50.62 ± 4.58</td>
</tr>
<tr>
<td>1 month post-operative</td>
<td>31</td>
<td>50.69 ± 4.2</td>
</tr>
<tr>
<td>3 months post-operative</td>
<td>31</td>
<td>50.11 ± 4.9</td>
</tr>
<tr>
<td>6 months post-operative</td>
<td>33</td>
<td>50.27 ± 4.6</td>
</tr>
<tr>
<td>12 months post-operative</td>
<td>22</td>
<td>49.8 ± 4.3</td>
</tr>
<tr>
<td>24 months post-operative</td>
<td>6</td>
<td>49.53 ± 4.6</td>
</tr>
</tbody>
</table>

Table 8-8: Mean average keratometry (D) and standard deviation in treated and control eyes at 1, 3, 6, 12 and 24 month reviews following collagen cross-linking. A decrease in mean average keratometry is noted over the review period in the treated eyes; however these data did not reach statistical significance.
At six months post-operatively 3 of the 33 (9.1%) eyes examined in the treated group had progressed (≥0.75D) by a mean of 1.14 ± 0.24D. At twelve months 2 of the 22 (9.1%) patients examined in the treated group showed progression from their pre-operative values by a mean of 0.78 ± 0.11D.

Significant increases in mean average keratometry were noted in the control eyes when compared to baseline values at three (0.35 ± 0.66 D, p<0.01), six (0.43 ± 0.83 D, p<0.01), and 12 months post-operatively (0.32 ± 0.92 D, p=0.02). At six months 12 of the 33 eyes (36.4%) examined in the control group had steepened (≥0.75D) by a mean of 1.29 ± 0.73 D. At twelve months, 5 of the 22 (22.7%) examined eyes in the control group had progressed (≥0.75D) by a mean of 1.45 ± 1.1 D.

<table>
<thead>
<tr>
<th>Review time point</th>
<th>Number of Subjects</th>
<th>Mean change in average K ± std (D) compared to pre-op</th>
<th>p value (Control vs. Treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treated eyes</td>
<td>Control untreated eyes</td>
</tr>
<tr>
<td>1 month post-operative</td>
<td>31</td>
<td>0.41 ±1.00</td>
<td>0.19 ±0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 months post-operative</td>
<td>31</td>
<td>-0.40 ±0.81</td>
<td>0.35 ±0.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months post-operative</td>
<td>33</td>
<td>-0.56 ±1.06</td>
<td>0.43 ±0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 months post-operative</td>
<td>22</td>
<td>-0.68 ±1.18</td>
<td>0.32 ±0.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 months post-operative</td>
<td>6</td>
<td>-1.40 ±0.13</td>
<td>0.58 ±0.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.01</td>
<td></td>
</tr>
</tbody>
</table>

Table 8-9: Change in average keratometry (D) compared to pre-operative values at the 1, 3, 6, 12 and 24 month follow up reviews. Statistics in columns two and three compare mean data to pre-operative values. Statistical values in column four compare the mean change in average keratometry between treated and control (untreated) eyes at each time point.
When analysing the average keratometry data using general linear mixed models accounting for random coefficient there was a difference (that approached significance) in the average keratometry over the follow up period between the treated and control eyes \((p=0.06)\). There were differences, once again approaching significance, in average keratometry between the treated and control eyes when accounting for the presence of eczema \((p=0.06)\) and asthma \((p=0.09)\). No significant interactions were noted between the treated and control group correlation outcomes over the follow up period when analysing for the presence of atopy (eczema \((p=0.28)\), asthma \((p=0.57)\), allergies \((p=0.73)\)), family history \((p=0.73)\), eye rubbing \((p=0.18)\) or severity of disease \((p=0.7)\).

### 8.4.3 Pentacam Simulated Corneal Astigmatism

Simulated corneal astigmatism values obtained from Pentacam analysis for each time point are detailed in Table 8-10 and Figure 8-9. When compared to pre-operative values a significant decrease in simulated corneal astigmatism in treated eyes was observed at 12 months post-operatively \((-1.00 \pm 2.12 \text{ D}, p=0.04)\), however, there was no significant change in this parameter at any other time point (Table 8-11). When compared to baseline, significant increases in simulated corneal astigmatism were observed in the control group at 12 and 24 months post-operatively \((0.39 \pm 0.93\text{D}, p=0.03 \text{ and } 0.37 \pm 0.46, p=0.04 \text{ respectively})\) (Table 8-11).

When analysing the simulated corneal astigmatism data using general linear mixed models accounting for random coefficient there was a difference that approached significance in the corneal astigmatism over the follow up period between the treated and control eyes \((p=0.06)\).

There were no significant interactions between the treated and control eyes over the follow up period when analysing for the presence of; atopy (eczema \((p=0.51)\), asthma \((p=0.45)\),
allergies (p=0.38)), family history (p=0.90), eyerubbing (p=0.53) and severity of disease (p=0.46).

<table>
<thead>
<tr>
<th>Review time point</th>
<th>Number of Subjects</th>
<th>Pentacam mean simulated corneal astigmatism ± std (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treated eyes</td>
</tr>
<tr>
<td>Pre-operative</td>
<td>38</td>
<td>6.07 ± 2.8D</td>
</tr>
<tr>
<td>1 month post-operative</td>
<td>31</td>
<td>6.21 ±2.7 D</td>
</tr>
<tr>
<td>3 months post-operative</td>
<td>31</td>
<td>6.19 ± 2.9D</td>
</tr>
<tr>
<td>6 months post-operative</td>
<td>33</td>
<td>5.96 ± 2.9 D</td>
</tr>
<tr>
<td>12 months post-operative</td>
<td>22</td>
<td>5.34 ± 2.0D</td>
</tr>
<tr>
<td>24 months post-operative</td>
<td>6</td>
<td>6.45 ± 2.8D</td>
</tr>
</tbody>
</table>

Table 8-10: Pentacam Simulated Corneal Astigmatism (D) at the 1, 3, 6, 12 and 24 month follow up reviews. When compared to pre-operative values a significant decrease in simulated corneal astigmatism in treated eyes was observed at 12 months post-operatively and significant increases in simulated corneal astigmatism in the control eyes at 12 and 24 months post-operatively.
<table>
<thead>
<tr>
<th>Review time point</th>
<th>Number of Subjects</th>
<th>Mean change in Pentacam Simulated corneal astigmatism ± std (D) compared to pre-op (Control vs. Treated)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treated eyes</td>
<td>Control untreated eyes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 month post-operative</td>
<td>31</td>
<td>0.37 ± 1.35&lt;br&gt;p=0.14</td>
<td>-0.02 ± 1.07&lt;br&gt;p=0.91</td>
</tr>
<tr>
<td>3 months post-operative</td>
<td>31</td>
<td>0.41 ± 1.31&lt;br&gt;p=0.09</td>
<td>0.11 ± 1.06&lt;br&gt;p=0.59</td>
</tr>
<tr>
<td>6 months post-operative</td>
<td>33</td>
<td>-0.03 ± 1.21&lt;br&gt;p=0.90</td>
<td>0.27 ± 1.28&lt;br&gt;p=0.23</td>
</tr>
<tr>
<td>12 months post-operative</td>
<td>22</td>
<td>-1.00 ± 2.12&lt;br&gt;p=0.04</td>
<td>0.39 ± 0.93&lt;br&gt;p=0.03</td>
</tr>
<tr>
<td>24 months post-operative</td>
<td>6</td>
<td>-0.63 ± 0.35&lt;br&gt;p=0.30</td>
<td>0.37 ± 0.46&lt;br&gt;p=0.04</td>
</tr>
</tbody>
</table>

Table 8-11: Change in Pentacam simulated corneal astigmatism (D) compared to pre-operative values at the 1, 3, 6, 12 and 24 month follow up reviews. Statistics in columns two and three compare mean data to pre-operative values. Statistical values in column four compare the mean change in the Pentacam simulated corneal astigmatism between treated and control (untreated) eyes at each time point.
Figure 8-7, Figure 8-8 and Figure 8-9: Trends in mean maximum keratometry, mean average keratometry and mean simulated corneal astigmatism (all vertical axes in dioptres) plotted against time along the horizontal axes including pre-operative values and at the major review intervals (1, 3, 6, 12 and 24 months) following corneal collagen cross-linking.
8.4.4 Central Corneal Thickness

Pentacam derived central corneal thickness (CCT) values for each time point are detailed in Table 8-12 and Figure 8-10.

CCT was significantly lower at all post-operative time points when compared to pre-operative measurements in the treated eyes (Table 8-13).

The mean CCT in the control eyes remained stable at 1, 3, 6, 12 and 24 months with values at no time point reaching significance when compared to baseline values (Table 8-13).

There was no significant difference in CCT between treated and control eyes pre-operatively (p=0.14). CCT however was significantly lower in the treated group compared to control eyes at one (p<0.01), three (p<0.01), six (p<0.01), and 12 months (p=0.04) (Table 8-13).

When analysing the central corneal thickness data using general linear mixed models accounting for random co-efficient there was a significant difference in CCT between treated and control eyes when analysing for disease severity (p=0.04); with the more diseased corneas having thinner corneas both before and after treatment.

When examining potential interactions over the follow up period after collagen cross linking, there were no statistically significant associations, however, those variables that approached statistical significance included the presence of asthma (p=0.09) and eye rubbing (p=0.06).
<table>
<thead>
<tr>
<th>Review time point</th>
<th>Number of Subjects</th>
<th>Mean central corneal thickness ± std (µm)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treated eyes</td>
<td>Control untreated eyes</td>
<td></td>
</tr>
<tr>
<td>Pre-operative</td>
<td>38</td>
<td>470 ± 29</td>
<td>480 ± 28</td>
<td></td>
</tr>
<tr>
<td>1 month post-operative</td>
<td>31</td>
<td>440 ± 35</td>
<td>487 ± 28</td>
<td></td>
</tr>
<tr>
<td>3 months post-operative</td>
<td>31</td>
<td>438 ± 425</td>
<td>476 ± 32</td>
<td></td>
</tr>
<tr>
<td>6 months post-operative</td>
<td>33</td>
<td>440 ± 31</td>
<td>480 ± 32</td>
<td></td>
</tr>
<tr>
<td>12 months post-operative</td>
<td>22</td>
<td>444 ± 40</td>
<td>486 ± 24</td>
<td></td>
</tr>
<tr>
<td>24 months post-operative</td>
<td>6</td>
<td>432 ± 43</td>
<td>491 ± 7</td>
<td></td>
</tr>
</tbody>
</table>

**Table 8-12**: Mean Pentacam derived central corneal thickness (µm) compared to pre-operative values at the 1, 3, 6, 12 and 24 month follow up reviews. CCT was lower at all post-operative time points when compared to pre-operative measurements in the treated eyes; however statistical analysis of these data did not reach statistical significance.
Table 8-13: Mean central corneal thickness (µm) in treated and control eyes measured with the Pentacam compared to pre-operative values at the 1, 3, 6, 12 and 24 month follow up reviews. Statistics in columns two and three compare mean data to pre-operative values. Statistical values in column four compare the mean change in central corneal thickness between treated and control (untreated) eyes at each time point.
8.4.5 Thinnest Point Pachymetry

Pentacam thinnest point pachymetry values for each time point are detailed in Table 8-14 and Figure 8-11. Thinnest point pachymetry values were significantly lower at all post-operative time points when compared to pre-operative values in treated eyes (Table 8-15).

The mean thinnest point pachymetry in the control eyes remained stable at the 1, 3, 6, 12 and 24 month time points, with no time point reaching statistical significance when compared to baseline values (Table 8-15).

<table>
<thead>
<tr>
<th>Review time point</th>
<th>Number of Subjects</th>
<th>Mean thinnest point pachymetry ± std (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treated eyes</td>
</tr>
<tr>
<td>Pre-operative</td>
<td>38</td>
<td>449 ± 32</td>
</tr>
<tr>
<td>1 month post-operative</td>
<td>31</td>
<td>417 ± 35</td>
</tr>
<tr>
<td>3 months post-operative</td>
<td>31</td>
<td>417 ± 40</td>
</tr>
<tr>
<td>6 months post-operative</td>
<td>33</td>
<td>411 ± 41</td>
</tr>
<tr>
<td>12 months post-operative</td>
<td>22</td>
<td>421 ± 41</td>
</tr>
<tr>
<td>24 months post-operative</td>
<td>6</td>
<td>419 ± 37</td>
</tr>
</tbody>
</table>

Table 8-14: Mean thinnest point pachymetry (µm) in treated and control eyes measured with the Pentacam compared to pre-operative values at the 1, 3, 6, 12 and 24 month follow up reviews. Thinnest point pachymetry values were significantly lower at all post-operative time points when compared to pre-operative values in treated eyes; however, these data did not reach statistical significance.
There was no significant difference in thinnest point pachymetry between treatment and control eyes pre-operatively (p=0.13). CCT was however significantly lower in the treatment group compared to control eyes at one (p<0.01), three (p<0.01), six (p<0.01), twelve (p=0.03) and 24 months post-operatively (p=0.04) (Table 8-15).

When analysing the thinnest point thickness data using general linear mixed models accounting for random coefficient there was no significant difference over the follow up period, or when accounting for any possible interactions.

<table>
<thead>
<tr>
<th>Review time point</th>
<th>Number of Subjects</th>
<th>Mean change in thinnest point pachymetry compared to pre-op thickness ± std (µm)</th>
<th>p value (Control vs. Treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treated eyes</td>
<td>Control untreated eyes</td>
</tr>
<tr>
<td>1 month post-operative</td>
<td>31</td>
<td>-33 ± 24</td>
<td>1 ± 13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>3 months post-operative</td>
<td>31</td>
<td>-32 ± 26</td>
<td>-3 ± 19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>6 months post-operative</td>
<td>33</td>
<td>-35 ± 32</td>
<td>-1 ± 16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>12 months post-operative</td>
<td>22</td>
<td>-24 ± 35</td>
<td>-3 ± 14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>24 months post-operative</td>
<td>6</td>
<td>-42 ± 15</td>
<td>-8 ± 17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.01</td>
<td></td>
</tr>
</tbody>
</table>

*Table 8-15:* Difference in thinnest point pachymetry (µm) measured with the Pentacam compared to pre-operative values at the 1, 3, 6, 12 and 24 month follow up reviews. Statistics in columns two and
Figure 8-10 and Figure 8-11: Trends in mean maximum central corneal thickness and mean thinnest point corneal thickness (all vertical axes in microns) plotted against time along horizontal axes, including pre-operative values and at the major review intervals (1, 3, 6, 12 and 24 months) following corneal collagen cross-linking. Both graphs highlight sustained reduction in Pentacam corneal thickness following treatment.
8.4.6 Corneal Densitometry

Corneal densitometry values were derived from Pentacam assessments for each time point and are detailed in Table 8-16. Post-operative/pre-operative corneal densitometry ratios for each time point are detailed in Table 8-17.

<table>
<thead>
<tr>
<th>Review time point</th>
<th>Number of Subjects</th>
<th>Mean Pentacam Corneal Densitometry ± std</th>
<th>p value (Control vs. Treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treated eyes</td>
<td>Control untreated eyes</td>
</tr>
<tr>
<td>Pre-operative</td>
<td>38</td>
<td>36.7 ± 12.6</td>
<td>32.2 ± 7.6</td>
</tr>
<tr>
<td>1 month post-operative</td>
<td>31</td>
<td>55.8 ± 17.0</td>
<td>34.1 ± 5.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>3 months post-operative</td>
<td>31</td>
<td>59.7 ± 16.5</td>
<td>35.5 ± 5.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>6 months post-operative</td>
<td>33</td>
<td>51.5 ± 15.2</td>
<td>35.6 ± 6.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>12 months post-operative</td>
<td>22</td>
<td>47.6 ± 10.2</td>
<td>36.1 ± 5.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>24 months post-operative</td>
<td>6</td>
<td>46.4 ± 7.4</td>
<td>37.47 ± 10.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.07</td>
<td></td>
</tr>
</tbody>
</table>

**Table 8-16**: Pentacam Corneal Densitometry values in treated and control eyes. Statistical values in columns two and three compare each time points mean to the pre-operative value. Statistical values in column four compare the mean change in Pentacam Corneal Densitometry between treated and control (untreated) eyes at each time point.

Compared to baseline measurements corneal densitometry post-operatively increased significantly in treated eyes at one (55.8 ± 17.0, p<0.01), three (59.7 ± 16.5, p<0.01), six
(51.5 ± 15.2, p<0.01) and 12 months (47.6 ± 10.2, p<0.01). By 24 months there was no significant difference in corneal densitometry compared to baseline (46.4 ± 7.4, p=0.07) (Table 8-16). There was no significant change in corneal densitometry in control eyes at any time point when compared to baseline values (Table 8-16).

<table>
<thead>
<tr>
<th>Review time point</th>
<th>Number of Subjects</th>
<th>Ratio of Pentacam Corneal Densitometry (post op/pre op)</th>
<th>p value (Control vs. Treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treated eyes</td>
<td>Control untreated eyes</td>
</tr>
<tr>
<td>1 month post-operative</td>
<td>31</td>
<td>1.6</td>
<td>1.1</td>
</tr>
<tr>
<td>3 months post-operative</td>
<td>31</td>
<td>1.7</td>
<td>1.1</td>
</tr>
<tr>
<td>6 months post-operative</td>
<td>33</td>
<td>1.5</td>
<td>1.1</td>
</tr>
<tr>
<td>12 months post-operative</td>
<td>22</td>
<td>1.4</td>
<td>1.2</td>
</tr>
<tr>
<td>24 months post-operative</td>
<td>6</td>
<td>1.4</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Table 8-17: Pentacam corneal densitometry ratio at 1, 3, 6, 12 and 24 month follow up appointments. Statistical values in column four compare the mean change in the ratio of Pentacam corneal densitometry between treated and control (untreated) eyes at each time point.

When analysing the corneal densitometry data using general linear mixed models accounting for random coefficient there was a difference approaching significance in the follow up period between the treated and control groups (p<0.01). A difference in corneal densitometry between the treated and control eyes was noted when accounting for the presence of allergies (p=0.05) over the follow up period. The correlation in the patients having allergy was $r= -0.38$ (SE 0.45), and those without $r= -0.62$ (SE 0.36). No other factors analysed had significant relevance interactions.
8.5 HIGHER ORDER CORNEAL ABERRATIONS

Corneal first surface wavefront aberrometry measured across a maximum diameter of 8mm and analysed up to 7th Zernike order revealed no significant change in spherical and higher-order aberrations in either the control or treated eyes across the entire follow up period.

8.6 OTHER PARAMETERS

Other corneal parameters examined that did not reach significance over the entire follow up period when examined using general linear mixed models included the I-S value (p=0.96), corneal volume (p=0.5), posterior best fit sphere (p=0.77), anterior best fit sphere (p=0.36), anterior elevation (p=0.11) and posterior elevation (p=0.54).

8.7 IN VIVO CONFOCAL MICROSCOPY

8.7.1 Corneal Basal Epithelium

The basal epithelial cells appeared subjectively enlarged and irregular in shape one week post-operatively in 15 of the 23 (65.2%) treated eyes that had images available for analysis (Figure 8-12). In one patient, nucleated basal epithelial cells were observed (Figure 8-13).

At 1, 3, 6 and 12 months post-operatively the basal epithelial cells had a normal subjective appearance in all patients (Figure 8-12)
Basal epithelial cells appeared subjectively enlarged and irregular in shape (imaged one week post-operatively). Normal appearance of basal epithelial cells at 1, and 12 months post-operatively (Image size 400 x 400µm).

Nucleated basal epithelial cells at one week in one patient (Image size 400 x 400µm)

8.7.2 Sub-Basal Nerve Plexus

The mean pre-operative sub-basal nerve density was $14.1 \pm 6.0 \text{ mm/mm}^2$ in treated eyes. One week postoperatively the sub-basal nerve plexus was absent in the central cornea in 16 of 22 corneas (72.7%) and appeared as fragmented remnants in 6 patients (27.3%) (Figure 8-15).
At one month the sub-basal nerve plexus was absent in 22 of the 31 corneas (71%) (Figure 8-15). Fragmented nerves were visible in 6 eyes (19.4%). Two corneas (6.5%) had significant corneal haze which obscured the sub-basal nerves.

At 3 months post-operatively continued absence of the sub-basal nerve plexus was noted in 15 of the 27 corneas imaged (55.5%) (Figure 8-15). Sub-basal nerve re-population was noted in 10 eyes (37.0%) with fragmented re-growth the common finding (Figure 8-15). Two eyes (7.4%) had developed significant corneal haze which obscured the sub-basal nerves.

At 6 months post-operatively fragmented regeneration of the sub-basal nerve plexus was observed in 17 of the 28 corneas (60.8%) (Figure 8-15). The sub-basal nerve plexus was absent in 5 corneas (17.9%) and appeared to have completely regenerated in 4 eyes (14%). One patient had poor quality images and was excluded from analysis.

At 12 months the sub-basal nerve plexus resembled the pre-operative state in 13 of the 15 (86.7%) corneas (Figure 8-15). Fragmentation of the nerve plexus was still visible in one cornea and continued absence of the sub-basal nerves was noted in one cornea.

Figure 8-14: A, B, Inflammatory cells (presumed antigen presenting cells) at the level of Bowman’s layer one week post-operatively. (Image size 400 x 400µm)
At 24 months post-operatively the sub-basal nerve plexus appeared completely reformed in all 8 eyes with available images (Figure 8-15).

Compared to baseline values (14.1 ± 6.0 mm/mm$^2$) the mean sub-basal nerve density had significantly decreased at one week (1.51 ± 2.3 mm nerve/mm$^2$, p<0.01) and at one (1.10 ± 2.5 mm nerve/mm$^2$, p<0.01), three (4.22 ± 6.23mm nerve/mm$^2$, p<0.01) and 6 months (7.08 ± 5.4 mm nerve/mm$^2$, p<0.01) post-operatively. There was no significant difference in sub-basal nerve density from pre-operative values at 12 months (13.32 ±5.32 mm nerve/mm$^2$, p =0.57) or 24 months (14.4 ±10.2 mm nerve/mm$^2$, p=0.66) (Table 8-18).

Analysis of inter-examiner repeatability revealed 95% limits of agreement of ± 9.1% for the sub-basal nerve density measurements. The intra-examiner repeatability revealed limits of agreement of ± 5.1%.

At 1 month post-operatively inflammatory cells were visible at the level of Bowman’s layer in 5 eyes (12.9%).(Figure 8-14). At 3 months 2 of the 25 (8%) patients who had images available for analysis exhibited persistent inflammatory cells at the level of Bowman’s layer. At 6 months following treatment persistent inflammatory cells were visible in only 1 (4.8%) patient at the level of Bowman’s layer.
Chapter 8

Figure 8-15 A, B. In vivo confocal microscopy images of the normal pre-operative sub-basal nerve plexus.

C. Absence of the sub-basal nerve plexus at one week post-operatively. D. Fragmentation of the sub-basal nerve plexus visible one month post-operatively.

E, F. Absence of the sub-basal nerve plexus one month post-operatively.

G. Continued reduction in the sub-basal nerve plexus visible at three months post-operatively. H. Fragmented regeneration of the sub-basal nerve plexus visible at three months post-operatively.

I, J. Fragmented regeneration of the sub-basal nerve plexus visible at six months post-operatively.

K, L, M, N. The sub-basal nerve plexus resembled the pre-operative state at 12 (K, L) and 24 (M, N) months post-operatively. (Image size 400 x 400µm)
8.7.3 Anterior Stroma

Anterior stromal oedema with hyper-reflective cytoplasm and extra cellular lacunae in a "honey comb" like appearance (Figure 8-16) was visible in all eyes at one week postoperatively (22 of 22 patients) with either reduced numbers or a complete absence of keratocyte nuclei. The anterior stromal oedema extended to a mean corneal depth of 261 ± 74µm. In 6 of the 22 (27%) corneas the keratocyte nuclei immediately posterior to Bowman's layer had a fragmented appearance (Figure 8-16). However, immediately posterior to the zone of anterior stromal oedema the keratocyte population and density appeared normal. In 15 of the 22 eyes (68.2%), although fine elongated needle like opacities were visible between depths of 284 to 366µm (Figure 8-16). Hyper-reflective bands were visible immediately posterior to these needle like opacities in 6 patients (27.3%) (Figure 8-17) (Figure 8-18). These bands extended from depths of 376 to 450µm.

At one month irregular dense hyper-reflectivity was observed immediately posterior to Bowman's layer in three eyes (10.7%) (Figure 8-18). Persistent anterior stromal oedema with a complete absence of keratocyte nuclei was noted in 24 of 28 corneas (85.7%) (Figure 8-18). Fragmented keratocytes in the anterior stroma were visible in 4 eyes (16%) (Figure 8-18) and one eye had a normal anterior stromal appearance. Three patients exhibited significant corneal haze that prevented clear imaging.

The anterior stromal oedema extended to a mean depth of 230 ± 66.5 µm at one month post-operatively (Figure 8-18). In the mid stroma an increased presence of elongated needle like structures (Figure 8-18) was observed in 13 eyes (46%) associated with what appeared to be fragmented keratocytes. These structures were visible between depths of 297 to 338 µm. Immediately posterior to the elongated keratocytes a transition to large hyper-reflective bands was observed in 6 corneas (21.4%) (Figure 8-17) (Figure 8-18). These bands were visible between depths of 298 to 362 µm.
Figure 8-16: A, B. Anterior stromal oedema with hyper-reflective cytoplasm and extracellular lacunae (honey comb like appearance) one week post-operatively. C, D. Fragmented keratocyte nuclei immediately posterior to Bowman\'s layer one week post-operatively. E, F. Fine elongated needle like opacities one week post-operatively. (Image size 400 x 400µm)

Three months post-operatively an area of dense hyper-reflectivity immediately posterior to Bowman\'s layer was noted in 10 of the 27 (40%) patients with available IVCM imaging. Anterior stromal haze and oedema (Figure 8-18) was still visible in 14 patients (51.8%) extending to a mean depth of 160 ± 63µm. Scattered re-population of keratocytes in the mid stroma was observed in 13 patients (48%) (Figure 8-18). Normal keratocyte appearance and density were seen at depths from 250-300µm. A visible transition zone was present in 17 corneas (63%) extending from a mean depth of 279 ± 56 µm with needle like\elongated keratocytes visible (Figure 8-18). The transition zone appeared to represent the demarcation between treated and un-treated corneal stroma; where normal keratocytes transitioned into needle like\elongated keratocytes and then transitioned into an area of
large hyper-reflective stromal bands. Large hyper-reflective bands (Figure 8-18) immediately posterior to the transition zone were visible in 14 corneas (51.8%) ranging in depth from 318 to 382 µm. In the stroma beneath this layer apparently normal keratocyte populations were visible.

![Figure 8-17: A, B, C Large hyper-reflective bands observed in the mid-stroma of the cornea visible at 1, 3 and 6 months post-operatively.](image)

At six months post-treatment persistent hyper-reflectively of the anterior stroma was visible in only one cornea (4.8%). Keratocyte repopulation of the anterior and mid-stroma was observed in the majority of eyes with only 6 of the 28 (21.4%) corneas with stromal imaging available having persistent microstructural oedema (Figure 8-18). This extended to a mean depth of 155 ± 35 µm. The visible transition zone with elongated and fragmented keratocytes was evident in 16 (57.1%) corneas (Figure 8-18) commencing at a mean depth of 277 ±65 µm and terminating at an mean depth of 338 ±59 µm. Persistent hyper-reflective bands remained in the mid stroma in 5 eyes (17.8%) (Figure 8-18). These ranged in depth from 320 µm to 381 µm.

At 12 months there was persistent hyper-reflectively of the anterior stroma in 2 eyes (13%). In all corneae the anterior stroma had re-populated with keratocytes and there was no residual stromal oedema (Figure 8-18). The mid-stroma showed transition zone remnants.
in 9 eyes (60%) with elongated and fragmented keratocytes (Figure 8-18) ranging in mean depth from 258µm to 303µm. Immediately posterior to these structures, large hyper-reflective bands were identified in 7 cornea (46.7%) (Figure 8-18, CC) ranging in depth from 305 µm to 368 µm.

At 24 months post-operatively the anterior corneal stroma was unremarkable in all patients (Figure 8-18). The remnants of the transition zone in the mid stroma (Figure 8-18) was visible in 3 corneas (37.5%) this extended from a mean depth of 260 ± 14µm and terminated at a mean depth of 325 ± 35µm. Persistent hyper-reflective bands were visible in 1 cornea.

The mean pre-operative anterior keratocyte density was 327 ± 165 cells/mm². When compared to baseline readings the mean anterior keratocyte density was significantly reduced at 1 week and 1, 3 and 6 months post-operatively with densities of 62.1 ± 89 cells/mm² (p<0.01), 77 ± 107 cells/mm² (p<0.01), 163 ± 142 cells/mm² (p<0.01), and 221 ± 95 cells/mm² (p<0.01) respectively (Table 8-18).

Compared to baseline values there was no significant difference in anterior keratocyte density at 12 (330 ± 137 cells/mm², p= 0.57) or 24 months (356 ± 44 cells/mm², p=0.8) (Table 8-18).

Analysis of inter-examiner repeatability of anterior stromal keratocyte density revealed 95% limits of agreement of ± 8.37%. The intra-examiner repeatability revealed 95% limits of agreement of ± 4%.
8.7.4 Posterior Stroma

The pre-operative posterior stromal keratocyte density was 197 ± 74 cells/mm². The posterior stroma remained unaffected at all-time points with no significant difference in keratocyte density at any time point when compared to pre-operative values (Table 8-18).

Significant corneal haze prevented clear imaging of the posterior corneal stroma in 4 corneas (18.2%) at one week; 1 patient (3.6%) at one month, 5 patients (18.5%) at three months, 6 patients (21%) at six months and one patient (6.7%) at 12 months.

Analysis of inter-examiner repeatability of posterior stromal keratocyte density revealed 95% limits of agreement of ± 3.98%. The intra-examiner repeatability revealed limits of agreement of ± 2.7%.

8.7.5 Endothelium

The mean pre-operative endothelial density was 2799 ±282 cells/mm². The endothelial density remained unaffected at all-time points, with no significant difference at any time point when compared to pre-operative measurements (Table 8-18).
Figure 8-18: *In vivo* confocal microscopy images of the corneal stroma imaged at 1 week, 1, 3, 6, 12 and 24 months following collagen cross-linking.

1 week post-operatively: A, B. Anterior stromal oedema visible at a depth of 93 and 120 µm respectively. C. Fragmented and elongated keratocytes visible at 182µm. D, E. Fragmented elongated ‘needle like’ structures visible at a depth of 221µm and 290µm respectively. F. Unaffected posterior stromal keratocytes at 352µm.

1 month post-operatively: G. Hyper-reflectivity immediately posterior to Bowman’s layer at 91µm. H. Clumped keratocyte nuclei at 130µm. I, J. Stromal oedema, with absence of keratocyte nuclei at 172µm and 230µm. K. Hyper-reflective ‘needle like’ stromal bands with absent keratocyte nuclei at 298µm. L. Hyper-reflective stromal bands with fragmented keratocyte nuclei at 355µm.
3 months post-operatively: M, N. Anterior stromal oedema at 69 and 150 µm respectively. O. Keratocyte nuclei with needle like protrusions visible. P, Q, R. Hyper-reflective needle like stromal bands at 242µm, 295µm and 330µm.

6 months post-operatively: S. Visible keratocyte nuclei at 91µm. T. Fragmented keratocyte nuclei at 140µm. U. Elongated keratocyte nuclei and fragmented keratocytes at 198µm. V, W, X. Elongated hyper-reflective needle like stromal bands visible at 244µm, 273µm and 360µm respectively.

12 month post-operatively: Y, Z. Anterior stromal keratocytes that resemble the pre-operative state at both 62µm and 110µm. AA. Elongated keratocyte nuclei at 198µm. BB, CC. Reduced density of hyper-reflective needle like bands visible at 230µm and 263µm respectively. DD. Keratocyte nuclei that resemble pre-operative state at 365µm.

24 months post-operatively: EE, FF. Anterior stromal keratocytes resembling the pre-operative state at 75µm and 99µm respectively. GG, HH, II, JJ. Mid stromal keratocytes, which resemble the pre-operative state. (Image size 400 x 400µm)
<table>
<thead>
<tr>
<th>Time Point</th>
<th>Number of Subjects</th>
<th>Mean Sub-Basal Nerve Plexus Density ± std (mm nerve/mm²)</th>
<th>Mean Anterior Stromal Keratocyte Density ± std (cells/mm²)</th>
<th>Mean Posterior Stromal Keratocyte Density ± std (cells/mm²)</th>
<th>Mean Endothelial Cell Density ± std (cells/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-operative</td>
<td>38</td>
<td>14.10 ± 6</td>
<td>327 ± 165</td>
<td>197 ± 74</td>
<td>2799 ± 282.2</td>
</tr>
<tr>
<td>1 week post-operative</td>
<td>28</td>
<td>1.51 ± 2.3</td>
<td>62 ± 89</td>
<td>180 ± 59</td>
<td>Not imaged</td>
</tr>
<tr>
<td>p&lt;0.01</td>
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<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
<td>p=0.76</td>
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<tr>
<td>1 month post-operative</td>
<td>31</td>
<td>1.10 ± 2.5</td>
<td>77 ± 107</td>
<td>198 ± 198</td>
<td>2605 ± 471.1</td>
</tr>
<tr>
<td>p&lt;0.01</td>
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<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
<td>p=0.67</td>
<td>p=0.20</td>
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<tr>
<td>3 months post-operative</td>
<td>31</td>
<td>4.22 ± 6.23</td>
<td>163 ± 142</td>
<td>198 ± 73</td>
<td>2604 ± 441.5</td>
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<tr>
<td>p&lt;0.01</td>
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<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
<td>p=0.47</td>
<td>p=0.70</td>
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<tr>
<td>6 months post-operative</td>
<td>33</td>
<td>7.08 ± 5.4</td>
<td>221 ± 95</td>
<td>193 ± 73</td>
<td>2748 ± 316.3</td>
</tr>
<tr>
<td>p&lt;0.01</td>
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<td>p=0.01</td>
<td>p=0.01</td>
<td>p=0.90</td>
<td>p= 0.33</td>
</tr>
<tr>
<td>12 months post-operative</td>
<td>22</td>
<td>13.32 ± 5.3</td>
<td>330 ± 137</td>
<td>190 ± 190</td>
<td>2373 ± 580.7</td>
</tr>
<tr>
<td>p=0.57</td>
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<td>p=0.57</td>
<td>p=0.57</td>
<td>p=0.71</td>
<td>p= 0.07</td>
</tr>
<tr>
<td>24 months post-operative</td>
<td>6</td>
<td>14.4 ± 10.2</td>
<td>356 ± 44</td>
<td>226.2 ± 54</td>
<td>Not Imaged</td>
</tr>
<tr>
<td>p=0.66</td>
<td></td>
<td>p=0.80</td>
<td>p=0.23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 8-18:** Sub-basal nerve plexus, anterior stromal, posterior stromal and endothelial cell densities compared to pre-operative values at the 1, 3, 6, 12 and 24 months follow up reviews. Statistical values in column 2-5 compare the mean values to the pre-operative values for each examined variable.
OCULAR RESPONSE ANALYSER

8.7.6 Corneal Hysteresis (CH)

CH values for each time point are detailed in Table 8-19. There were no significant differences in CH at 1, 3, 6 and 12 months compared to the pre-operative values for treated and control eyes. At 12 months, there was a significant reduction in CH in the control eyes (p=0.05). There was no significant difference in CH between the two groups pre-operatively and at any follow up time point (Table 8-19).

<table>
<thead>
<tr>
<th>Review time point</th>
<th>Number of Subjects</th>
<th>Mean corneal Hysteresis ± std (mmHg)</th>
<th>p value (Control vs. Treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treated eyes</td>
<td>Control untreated eyes</td>
</tr>
<tr>
<td>Pre-operative</td>
<td>38</td>
<td>7.7 ±1.3</td>
<td>7.7 ±1.1</td>
</tr>
<tr>
<td>1 month post-operative</td>
<td>31</td>
<td>7.9 ±1.5</td>
<td>7.6 ±1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.71</td>
<td></td>
</tr>
<tr>
<td>3 months post-operative</td>
<td>31</td>
<td>8.0 ±1.2</td>
<td>7.6 ±1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.30</td>
<td></td>
</tr>
<tr>
<td>6 months post-operative</td>
<td>33</td>
<td>7.8 ±1.8</td>
<td>7.4 ±1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.58</td>
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<tr>
<td>12 months post-operative</td>
<td>22</td>
<td>7.4 ±1.8</td>
<td>6.8 ±1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.40</td>
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</table>

Table 8-19: Mean corneal hysteresis compared to pre-operative values at the 1, 3, 6 and 12 months follow up reviews. Statistical values in columns two and three compares the mean values to the pre-operative values at each review time. Statistical values in column five compare the mean change in corneal hysteresis between treated and control (untreated) eyes at each time point.
When analysing the CH data using general linear mixed models accounting for random co-efficient no significant interactions were noted over time between any of the factors identified including time post-treatment.

8.7.7 Corneal Resistance Factor (CRF)

CRF values for each time point are detailed in Table 8-20. There was no significant change in CRF at any post-operative time point in the treated and control eyes. There was no significant difference in CRF between the groups pre-operatively and at any follow up time point (Table 8-20).

<table>
<thead>
<tr>
<th>Review time point</th>
<th>Number of Subjects</th>
<th>Mean Corneal Resistance Factor ± std (mmHg)</th>
<th>p value (Control vs. Treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treated eyes</td>
<td>Control untreated eyes</td>
</tr>
<tr>
<td>Pre-operative</td>
<td>38</td>
<td>6.0 ± 1.2</td>
<td>6.2 ± 1.4</td>
</tr>
<tr>
<td>1 month post-operative</td>
<td>31</td>
<td>6.0 ± 1.2</td>
<td>6.3 ± 1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 months post-operative</td>
<td>31</td>
<td>5.9 ± 1.1</td>
<td>6.4 ± 1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.86</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months post-operative</td>
<td>33</td>
<td>5.9 ± 1.2</td>
<td>6.2 ± 1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.39</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 months post-operative</td>
<td>22</td>
<td>5.6 ± 0.9</td>
<td>6.6 ± 1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.31</td>
<td></td>
</tr>
</tbody>
</table>

Table 8-20: Mean corneal resistance factor in treated and control eyes compared to pre-operative values at the 1, 3, 6, 12 and 24 months follow up reviews. Statistical values in columns two and three compares the mean values to the pre-operative values at each review time. Statistical values in column four compare the mean change in the corneal resistance factor between treated and control (untreated) eyes at each time point.
When analysing CRF data using general linear mixed models accounting for random co-efficient there were no significant interactions between any of the factors identified including time post-treatment. The presence of asthma had an effect that approached statistical significance (p=0.06), suggesting that those patients who had asthma had reduced CRF when compared to those without.
Chapter 9 : Discussion
9.1 Introduction

As noted in earlier chapters, until a little over a decade ago the principle management of keratoconus was the initial use of spectacles, followed by specialized contact lenses in the majority, with around 20% of those with keratoconus ultimately progressing to corneal transplantation. However, the first published clinical trial of corneal collagen cross-linking for the treatment of keratoconus, a case series of 22 subjects performed by Wollensak et al in 2003 in Dresden, Germany, awakened the international community to the potential of this novel, less invasive, surgical treatment for keratoconus. 1

Since that initial publication there has been a growing body of literature regarding clinical studies of this procedure. Unfortunately, the majority of these studies are case reports, retrospective studies or prospective observational series. Indeed, at the commencement of the current study of collagen cross linking that constitutes a large part of this thesis, there were no published, prospective, randomised, controlled trials (RCT). Table 9-1 provides a summary of the major studies 2000 - 2012. 1-14

Although collagen cross-linking for keratoconus has been increasingly adopted in Europe and elsewhere, surprisingly only two of the studies published to date are actually randomised control trials. 4,13 Thus, despite clinical acceptance of this technique by many ophthalmologists, the quality of the evidence base is limited and the variable construction of available trials makes comparisons between published data difficult. The current study was designed as a randomised trial as the authors believe this is essential in any prospective study of keratoconus, given the highly variable topographic and refractive features of the disease. In this chapter the outcomes of this RCT of collagen cross-linking for keratoconus are discussed in the context of the available literature.
<table>
<thead>
<tr>
<th>Reference, Year And Country</th>
<th>Study Design and Subjects</th>
<th>Mean follow up Months ± SD</th>
<th>Summary of Key Results</th>
</tr>
</thead>
</table>
| Wollensak et al 2003 ¹ Germany | Case series 23 eyes of 22 patients | 23.2 ± 12.9 Range 3 to 47 months | • Mean decrease in Max K of 2.01 ±1.74 D  
• Mean decrease in SE of 1.14 ± 2.18 D  
• Mean BCVA increased 1.26 ± 1.5 lines |
| Caporossi et al 2006 ² Italy | Prospective non-randomized case series 10 eyes of 10 patients | 3 months | • Decrease in Max K of 1.23 to 3.07 D  
• Mean decrease in Ave K 2.1 ± 0.13 D  
• Mean decrease in Min K of 2.4 ± 0.16 D  
• Mean BCVA increased 1.66 Snellen lines  
• Mean UCVA increased 3.6 Snellen lines |
| Raiskup-Wolf et al. 2008 ³ Germany | Retrospective study 241 eyes | 26.7 ± 16.2 Range 1-6 years | • BCVA ≥ 1 line in 57% of eyes and remained stable in 24% of eyes  
• Mean decrease in Max K by 2.21 D in 60% and stable in 19% of eyes  
• Mean decrease in Max K by 1.91 D in 54%, remained stable in 35% of eyes |
| Wittig-Silva et al 2008 ⁴ Australia | Prospective randomized controlled trial 33 eyes | Range 3-12 months | • Mean decrease Max K of 1.45 ± 1.00 D  
• Mean BCVA improvement 0.12 LogMAR |
| Agrawal 2009 ⁵ India | Retrospective case series 68 eyes of 41 patients | 10.0 ± 3.6 Range 6 to 16 months | • Mean Max K decrease of 2.47 ± 3.89D in 54% of eyes  
• BCVA improved by at least one line in 54% and remained stable in 28% |
<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Type</th>
<th>Eyes/Patients</th>
<th>Range Months</th>
<th>Max K Decrease</th>
<th>Ave K Decrease</th>
<th>BCVA Improvement</th>
<th>Other Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coskunseven 2009</td>
<td>Turkey</td>
<td>Prospective, controlled</td>
<td>38 eyes of 19 patients</td>
<td>9 ± 2</td>
<td>Mean decrease in Max K of 1.57 ± 1.14 D</td>
<td></td>
<td></td>
<td>Mean decrease in Min K of 0.03 ± 2.22 D, Mean BCVA improved 0.10 ± 0.14 LogMAR, Mean UCVA improvement of 0.06 ± 0.05 LogMAR</td>
</tr>
<tr>
<td>Fournie 2009</td>
<td>France</td>
<td>Prospective, uncontrolled, trial</td>
<td>20 eyes of 20 patients</td>
<td>Range 3-18 months</td>
<td>Mean decrease in Max K of 1.68 ± 2.18 D at 12 months</td>
<td>Mean decrease in Ave K decrease of 0.66 ± 2.4 D at 12 months</td>
<td>Mean BCVA improvement of at least one line in 56.25% of patients</td>
<td></td>
</tr>
<tr>
<td>Grewal 2009</td>
<td>India</td>
<td>Prospective, uncontrolled, trial</td>
<td>102 patients</td>
<td>12 months</td>
<td></td>
<td></td>
<td></td>
<td>No significant change in BCVA, spherical equivalent or cylinder vector</td>
</tr>
<tr>
<td>Hoyer 2009</td>
<td>Germany</td>
<td>Retrospective series</td>
<td>153 eyes of 111 patients</td>
<td>Range 12-36 months</td>
<td>Mean decrease Max K of 4.34 D at 3 yrs</td>
<td>Mean improvement in BCVA of 1 line or more in 60.6% of patients at 3 years</td>
<td>Progression of keratoconus in 3 patients</td>
<td></td>
</tr>
<tr>
<td>Koller 2009</td>
<td>Switzerland</td>
<td>Prospective, controlled</td>
<td>21 patients</td>
<td>12 months</td>
<td>Mean decrease Min K of 0.62 D at 1 year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vinciguerra 2009</td>
<td>Italy</td>
<td>Prospective, controlled</td>
<td>28 eyes</td>
<td>12 months</td>
<td>Mean decrease in Max K of 6.16 D</td>
<td>Mean decrease in Ave K of 6.07 D</td>
<td>Mean decrease in SE of 0.43 D</td>
<td>Mean improvement BCVA 0.14 LogMAR</td>
</tr>
</tbody>
</table>
### Table 9-1: Published human clinical studies of corneal collagen cross-linking in the treatment of keratoconus presented in chronological order with summary of key observations.

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Country</th>
<th>Duration</th>
<th>Key Observations</th>
</tr>
</thead>
</table>
| Mazzotta 2010    | Prospective, uncontrolled, trial | Italy   | 12 months | - Mean decrease in mean $K$ of 1.3D  
- Mean BCVA improved $0.75 \pm 0.08$ Snellen lines.  
- UCVA improved $0.51 \pm 0.11$ Snellen lines. |
| Hersh 2011       | Prospective randomized controlled trial | USA     | 12 months | - Mean decrease in Max $K$ of 2.0D and mean decrease in Ave $K$ of 1.50D  
- Mean decrease in Min $K$ of 1.2D  
- BCVA improved $0.13 \pm 0.21$ LogMAR  
- Loss of BCVA by 2 or more Snellen lines in 6 eyes (8.5%) |
| Kanellopoulos 2012 | Prospective randomized comparative case series | Greece | Minimum of 28 months | - Mean UCVA improved 20/60 to 20/40  
- BCVA improved from 20/30 to 20/25  
- Mean decrease in sphere by 2.4 D  
- Mean decrease in cylinder by 2.9 D  
- Mean decrease Max $K$ of 49.5 to 46.1 D |

**Table abbreviations**
- Max $K$= Maximum Keratometry, Min $K$= Minimum Keratometry, Ave $K$= Average Keratometry, BCVA= Best Corrected Visual Acuity, UCVA= Uncorrected Visual Acuity, SE= Spherical Equivalent, D= Dioptres

### 9.2 UNCORRECTED VISUAL ACUITY AND BEST CORRECTED VISUAL ACUITY

The current study showed no statistically significant change in the uncorrected visual acuity (UCVA or vision) in control eyes at any time point. In contrast, one non-randomised study
reported a small but significant decrease in UCVA in control eyes (mean decrease of 0.08 ± 0.12 LogMAR) at 9 months.  

In the current study there was no statistically significant change in UCVA in treated eyes in the early post-operative period (one month post-operatively. However, there were statistically significant improvements in UCVA as the study progressed at the 3, 6, 12 and 24 month post-operative time points. None the less it must be stated that a limitation of the study was the lack of examiner masking in terms of visual acuity measurements.

UCVA has previously been reported to be reduced in eyes treated with corneal cross-linking at one month post-operatively; however, this did not reach significance and was attributed to corneal haze. Significant improvements in UCVA have been reported to occur between 6 months and 1 year following treatment which concurs with the results of the current study.

The current study identified stability in the best corrected visual acuity (BCVA) in both the treated and control eyes. In contrast a clinical trial performed in Germany reported an improved BCVA in 65% of patients by a mean of 1.26 Snellen lines. Other studies have reported significant improvements in BCVA between 6 to 12 months post-operatively with mean gains of between 1.26 to 1.66 Snellen lines. Nonetheless, none of these studies were randomised controlled trials, so the results cannot be confidently compared with the results of the current study.

Grewal et al reported stability in BCVA over the entire one year follow up period following cross-linking, whereas other studies report improved acuity in the majority of subjects, stable acuity in the remainder of subjects, and significant decreases in the BCVA in the untreated eye. However, the majority of these studies were non-randomised control trials so apparent stability, due to coincidence, in a relatively slowly advancing disease
cannot be confidently excluded. Interestingly, in a randomised control trial by Wittig-Silva et al a trend towards improving BCVA was observed in the experimental group but this did not reach significance at 6 to 12 month post-operatively. This is in agreement with the results of the current RCT.

Both of these Australasian randomised control trials have therefore shown that collagen cross-linking does not significantly affect BCVA in the first 12 months post-operatively.

9.3 REFRACTIVE ERROR

Statistical analysis in the current study highlighted stability in the spherical equivalent refraction over all post-operative time points in both the control and treated eyes.

Improvements in refractive error following collagen cross-linking have been highlighted in several published reports. Indeed, mean improvements of 1.03 – 2.25 diopters spherical equivalent over 6 to 23 months have been reported in non-randomised, un-controlled, clinical trials. In contrast, the randomised control trial by Wittig-Silva et al. noted no significant change in the refractive sphere, astigmatism or manifest refraction spherical equivalent (MRSE) values at 6 and 12 months - in either treated or control eyes. These results are similar to the outcomes of the current study. Both RCT studies therefore suggest that corneal collagen cross-linking results in stability, but not an improvement (reduction) in spherical equivalent refractive error over 12 to 24 months post treatment.

9.4 CORNEAL TOMOGRAPHY

In the current study 14 control eyes (42.4%) demonstrated progression (≥0.75D) in mean maximum keratometry by a mean of 1.82D at six months, whereas, by 12 months seven
control eyes (32%) had progressed by a mean of 2.3D. It must be noted in relation to the modest difference between the values at 6 and 12 months, that the fellow eye of 13 subjects (33%) was treated with cross-linking (at least six months after the initial treatment) when further significant progression was noted during the follow-up period.

These results, highlighting rapidity of change in keratoconic corneas, echo other studies where significant keratometric progression of keratoconus in the control eyes has been observed - even over relatively short study periods. 1, 4, 17 Notably, in the original study of cross-linking, Wollensak et al 1 noted that 22% of control eyes showed progression by an average of 1.48D at 12 months post-operatively.

When compared to baseline, significant increase in simulated corneal astigmatism of 0.39D was observed in the control group at 12 months. These data reflect, although of greater magnitude, the results of Vinciguerra et al. 11 who reported increases in the simulated corneal astigmatism of 0.14D at 12 months in control eyes. 11

It can therefore be concluded that the natural history of keratoconus, over relatively short study periods, involves significant progression in terms of keratometry and corneal astigmatism. This progression appears readily quantifiable within 12 months or less in those (younger) patients who are eligible for collagen cross-linking.

The current study highlighted an early, somewhat paradoxical, corneal steepening in the treated eyes at one month post-operatively. However, there was a subsequent decrease in both the average keratometry and the mean maximum keratometry at 3 months, 6 months, and 24 months post-operatively. This early corneal steepening in treated eyes has also been reported in other studies. 12, 13 This counter-intuitive initial steepening has been postulated to be largely artifactual as a result of tomographic imaging artefacts secondary to corneal haze following treatment. 18
Corneal topographers using Placido elements analyse light reflected from the cornea to extrapolate data regarding the corneal power and anterior corneal surface. These systems assume standard physiological refractive indices of the cornea (1.3376), and therefore alterations in the corneal architecture due to corneal oedema, haze, and collagen synthesis may alter the refractive index of the cornea, consequently affecting the extrapolated calculations. An alternative theory suggests that the removal and subsequent healing of the corneal epithelium may affect the total corneal power. This difference is potentially caused by a thinner epithelium at the cone apex following re-epithelialisation where the stroma continues to be steeper. This may explain the apparent topographic steepening.

Corneal “flattening” has been reported in many studies of corneal cross-linking while others have reported stability in keratometry. The mean keratometry has been reported to decrease by between 0.66D and 2.66D 12 months following treatment. This agrees with the outcomes from the current study with 77% of treated eyes exhibiting flattening of average keratometry at 12 months. Wollensak et al similarly noted that 70% of their subjects showed flattening of average keratometry at 12 months post-operatively.

The progressive nature of the decrease in keratometry was also reported by Raiskup-Wolf et al. who reported decreases in apical keratometry of 2.21D and 4.84D at two and four years after treatment respectively.

In agreement with other studies, a significant decrease in simulated corneal astigmatism was observed in treated eyes in the current study at 12 months post-operatively.

In terms of ongoing progression of keratoconus despite cross-linking, the current study noted 4 treated eyes (12%) had progressed by a mean of 2.4D at 6 months, and 2 eyes
(9.1%) had progressed by a mean of 1D at 12 months post-operatively. Ongoing progression (increase) of keratometric values of treated eyes has been reported to occur in a small number of eyes following cross-linking in a number of studies. The reported incidence of continued progression following cross-linking is highly variable ranging from 0 to 7.6%.

9.5 ANTERIOR AND POSTERIOR ELEVATION

In agreement with the report of Henriquez et al.\textsuperscript{16} the current study noted no significant change in anterior and posterior corneal elevation and the anterior and posterior best fit sphere in either the treated or control eyes over the post-operative period. However, another, non-randomised study has reported decreased anterior corneal elevation from three months post-operatively although the study design does not enable direct comparisons\textsuperscript{12}

Koller et al.\textsuperscript{10} reported a significant decrease in the index of height asymmetry; index of surface variance and keratoconus index following treatment compared to the contralateral "control" eye. These data tend to suggest that an increase in corneal regularity occurs following cross-linking.\textsuperscript{10} However the current study, which in terms of regularity only investigated changes in the I-S value, revealed no significant change in either the control or treated eyes.

9.6 CORNEAL THICKNESS

In the current study there was no significant change in either the central corneal thickness (CCT) or thinnest point pachymetry (TPP) in control eyes over the study period.
Interestingly, a recent study by Choi et al. noted that in contrast to keratometric changes, CCT and TTP were not significant indicators for keratoconus progression. In relation to treated eyes, significant decrease in both CCT and TPP was noted at 1 and 3 months post operatively in the current study. This decrease appeared to stabilise at 6 months post-operatively but was still reduced at 24 months when compared to pre-operative values. Other studies assessing changes in corneal thickness after collagen cross-linking have noted transient increase in thickness immediately after treatment or, more commonly, transient decrease in thickness occurring one month after treatment. Other, non-randomised studies, have noted no post-operative changes in Pentacam derived CCT measurements.

Postulated explanations for this decrease in CCT have included: altered hydration patterns of the cornea; increased pump-site activity/turnover following hypoxia, UV stress, epithelial thinning; and keratocyte loss from the anterior mid stroma. The major alternative hypothesis purports that inherent instrument limitations combined with changes in the optical properties of the cornea following the treatment may be largely responsible for corneal thickness measurements being artifactually low. It has also been suggested that the stromal demarcation line may influence the optical measurement of the posterior surface of the cornea due to increased reflectivity.

Corneal tomographers such as the Pentacam and Orbscan rely on light scatter to measure corneal thickness and the reported decrease in CCT measured by Orbscan II, in eyes that have undergone PRK, is considered to be due to corneal haze. Carporossi et al. noted that the Orbscan II tomographer significantly underestimated corneal thickness compared to ultrasound pachymetry following collagen cross-linking. Crucially, this study reported that when using ultrasound pachymetry there was no difference in the CCT at any time point following the treatment compared to pre-operative values. Other studies have
reported that the corneal pachymetry returns to pre-operative values by 3 months post-operatively when measured by ultrasound pachymetry, and by 12 months post-operatively when measured by Orbscan II.

In contrast, studies using the Pentacam method have revealed consistently lower CCT measurements over the entire 12 month follow up period following cross-linking. These published reports reflect the outcomes noted in the current study where Pentacam pachymetry measures were employed.

In summary, the author believes that on balance the available evidence suggests that the apparent decrease in corneal thickness noted in the current study is most likely to be due to changes in the optical properties of the cornea due to persistent haze that occurs following collagen cross linking.

### 9.6 OCULAR RESPONSE ANALYSER

Perhaps surprisingly, but in agreement with other published studies, CH and CRF were shown to be unaffected by corneal collagen cross-linking in keratoconus. Our observations and those of others therefore suggests that the increase in mechanical rigidity that is well documented in ex vivo studies is not detectable in-vivo using the ORA system. Spoerl et al., in trying to extract meaningful information from ORA data, noted that the area under peak 2 appears to be a more sensitive parameter to detect biomechanical changes after collagen cross-linking than CH or CRF alone, however, this method of analysis is not currently commercially available for assessment.
9.7 WAVEFRONT ANALYSIS

Corneal wavefront analysis revealed that spherical and higher-order aberrations did not show significant variations in the follow-up period in either treated or control eyes. Although this has also been confirmed by the results of some studies, others have described decrease in the coma component of the ocular aberrations at one and six months post-operatively. The differences in these results compared to the current studies may reflect differences in instrumentation as other studies used the Orbscan II tomographer and the Keratron Scout topographer (Optikon, Italy).

9.8 CLINICAL OUTCOMES

All, except one patient, experienced moderate to severe pain for the first 72 hours following the treatment due to the corneal epithelial defect. Some authors have suggested altering the cross linking protocol to maximize patient comfort by performing the procedure without epithelial removal. However, assessment of the penetration of riboflavin into the corneal stroma showed that neither superficial epithelial trauma nor tetracaine administration alone was sufficient to allow the penetration of riboflavin into the corneal stroma. Notably, without the presence of a photosensitizer (such as riboflavin) in the cornea, light at wavelengths of 350 nm and an irradiance of 3 mW/cm² will cause damage to the corneal endothelium. Other laboratory studies have noted significant improvement in the penetrance of riboflavin after the administration of benzalkonium chloride to the cornea.

A study investigating the effects of cross-linking in rabbit eyes reported a smaller increase in Young’s modulus in eyes with intact epithelium compared to that in eyes following epithelial removal (21.30% and 102.45% respectively). The weaker biomechanical effect of cross-linking treatment with intact epithelium is presumably due to insufficient and
inhomogeneous trans-epithelial riboflavin diffusion into the stroma. \(^{41}\) The intact epithelium acts as a barrier that slows the absorption of riboflavin (molecular weight, 376.37 g/mol) into the cornea leading to poor and incomplete penetration. \(^{42}\)

A common outcome in the current study was the development of both corneal haze and a stromal demarcation line (occurring in 76% of patients at one month post-operatively). The stromal demarcation line is thought to represent the transition line between the cross-linked anterior corneal stroma and untreated posterior corneal stroma. \(^{31}\) Seiler and Hafezi \(^{31}\) showed that the demarcation line is detectable by slit-lamp examination as early as two weeks following treatment and present over a total follow up period of six months at a depth of approximately 300 µm (or 60%) of the corneal thickness. \(^{31}\) Similarly, Koller et al.\(^{10}\) noted that the stromal demarcation line occurred at 50% – 80% of corneal depth. They also reported the gradual anterior movement and fading of the demarcation line within the corneal stroma over the 12 month follow up period.

The persistence of stromal demarcation lines was shown to be variable in the current study, with some resolving by six months post-operatively and others still being visible at one year post-operatively. However, the presence of corneal haze and the related demarcation line was not usually associated with clinical symptoms. Nonetheless, in three patients there was persistent light sensitivity attributed to corneal haze with associated inflammation which required a short course of topical corticosteroids.

The current study quantified corneal haze using Pentacam Sheimpflug densitometry. Densitometry values peaked at 3 months post-operatively and slowly decreased to pre-operative values at 24 months post-operatively. These longer term trends tend to agree with a similar study investigating haze using densitometry measurements assessed by Pentacam in combination with slit-lamp assessment. \(^{43}\) However, the study by Greenstein
et al. noted that densitometry peaked at one month with little change by three months and had not returned to baseline by 12 months in the treated eyes. 

Interestingly, the current study also noted that significantly higher densitometry values occurred in those patients with ocular allergies compared to those without. Posterior stromal haze was noted in five patients (12.8%) at 1 - 3 months post-operatively, however, the posterior stroma and endothelium immediately posterior to the haze was clinically unremarkable.

A small case series reported by Lim et al noted late onset stromal scarring at a depth of approximately 300µm in 2 of 30 patients treated (7%). This was first evident at three-months post-operatively when dense, deep, paracentral stromal scarring was noted adjacent to the apex of the cone. This was also associated with decreased best corrected visual acuity. In vivo confocal microscopy revealed reduced keratocyte density and dense hyper-reflective bands in a reticular pattern associated with the area of scarring. The keratocyte nuclei assumed attenuated, elongated forms, which the authors suggest may have been indicative of fibroblastic transformation, however, the deep stroma and endothelium posterior to the scar were normal. Hafezi et al presented a case following a repeat treatment with cross-linking, in which stromal fibrosis at a depth of 160-250 µm was observed.

The reasons for the variability in the development of haze following cross-linking remain unclear. Suggested associations include higher pre-operative keratometric values and thinner corneal pachymetry prior to treatment. Higher rates of complications such as corneal scarring have been associated with subjects aged over 35 years and with corrected visual acuity of less than 20/25 (6/7.5).
9.9 IN-VIVO CONFOCAL MICROSCOPY

9.9.1 Epithelium

In the current study there were no significant alterations in corneal epithelial cell morphology following the treatment. Mild enlargement of the basal epithelial cells was noted at one week post-operatively which is simply thought to be indicative of the healing process.\textsuperscript{19} Mazotta et al.\textsuperscript{47} qualitatively analysed epithelial changes in 44 cross-linked keratoconic corneas using laser scanning in-vivo confocal microscopy. There was no gross damage to the limbal region, although mild hyper-reflectivity of the extracellular tissue surrounding the palisades of Vogt was observed. In agreement with the results of the current study, a qualitative improvement in the cell mosaic of the corneal epithelium (when compared to the pre-operative state) was noted, especially in the region of the corneal apex, a region where the epithelium is often thin with poorly defined basal epithelial cell borders and distorted cell shape.\textsuperscript{47}

9.9.2 Sub-Basal Nerve Plexus

The current study is the first to quantify the changes in sub-basal nerve density following collagen cross-linking. Significant reduction in the sub-basal nerve density was observed between 1 week and 6 weeks post operatively, thereafter, regeneration started between the second and third post-operative months. It was observed that the sub-basal nerve density eventually returned to pre-operative levels at 12 months. These data generally agree with existing data in the published literature.\textsuperscript{4,28,48} Indeed, the majority of studies have reported a complete disappearance of the sub basal nerve plexus in the central cornea in the early post-operative stages (2 - 4 weeks),\textsuperscript{28,48,49} with subsequent (complete) nerve plexus
regeneration by 12 - 24 months after treatment. The sub-basal nerve plexus in the peripheral cornea outside the treatment zone has been reported to remain unaffected. Decreased sub-basal nerve fibre density has also been reported to occur in the immediate post-operative period following photorefractive keratectomy (PRK). Initial regeneration following PRK has been reported to occur as early as 4 weeks post operatively, however the sub-basal nerve density has been shown to be reduced in the first 12 months post operatively with a return to baseline values taking up to 24 months. This suggests that the nerve regeneration following cross-linking follows a similar, though perhaps slightly shorter time course, to that observed following PRK.

### 9.9.3 Corneal Stroma

The anterior (50 to 120 µm) and mid (120 to 300 µm) corneal stroma exhibited signs of (microstructural) oedema in the current study. This resulted in a honeycomb-like appearance on in-vivo confocal microscopy with an absence of keratocytes. This microstructural response parallels observations in other published clinical reports. Absence of keratocyte nuclei in the early post-operative period has been reported, extending to depths of between 200 µm and 340 µm from Bowman's layer. In the current study the depth to which the anterior stromal oedema and accompanying absence of keratocyte nuclei extended, progressively decreased over the follow up period, returning to pre-operative appearances at 12 months.

Apoptosis is known to occur in the period immediately following injury to the corneal epithelium, Bowman's layer and anterior stroma, following surgical events such as photorefractive keratectomy (PRK). Studies of simple corneal epithelial scrape injury without PRK in rabbit eyes revealed that keratocyte apoptosis was confined to the superficial stroma - extending to a depth of 50-75µm. Although anterior stromal oedema and keratocyte loss may be attributed to injury to the epithelium, Bowman's layer and
anterior stroma, recent ex-vivo studies have demonstrated that standard corneal cross-linking (with epithelial removal) triggers more anterior keratocyte apoptosis than corneal epithelial removal with either UVA or riboflavin alone. 53

The cytotoxic level of UVA irradiance for keratocytes is 0.5 - 0.7mW/cm² in the anterior 50µm of the corneal stroma. 54 Corneal collagen cross-linking with riboflavin causes dose dependent keratocyte damage in human corneas down to a depth of 300um using the standard recommended surface UVA dose of 5.4 J/cm². 54 Theoretically this results in apoptosis of the “unhealthy” keratocytes in the anterior corneal stroma 21 and secondarily acts to stimulate repopulation with “healthier” cells. Studies have noted a clear transition area between the oedematous hypo-reflective stroma in the early post-operative period which contains visible keratocyte apoptotic bodies. 47

In the current study three patients were noted to have hyper-reflective granular deposits beneath Bowman’s layer in the early post-operative period. These were similar to those observed by Croxatto et al. 48 A recent study investigating in-vivo confocal microscopy changes in chronic corneal oedema revealed similar anterior stromal granularity which was either finely or coarsely hyper-reflective. 55 This suggests that the granular deposits observed in the current study may be associated with the microstructural oedema that occurs following cross-linking treatment.

The current study noted elongated ‘needle like’ structures at depths of 284 to 366µm at one week which persisted up to 12 months post operatively. These structures appeared to be mainly located at the transition zone between the treated and untreated corneal stroma, with larger hyper-reflective bands visible immediately posterior to them. The larger hyper-reflective bands have been noted in previous reports in the anterior-mid stroma. 4, 48, 56
The hyper-reflectivity of the extra-cellular matrix and visualisation of what has been described as ‘activated keratocyte nuclei’ have been noted from the third to sixth month post-operatively. This hyper-reflectivity has been suggested to represent stromal compaction by new structured collagen fibres in the anterior-mid stroma after cross-linking. These changes have been documented up to at least two years post operatively.

The depth to which the larger, deeper, hyper-reflective bands extended in the current study appeared to remain stable after the first month, and were absent in all except one patient at 2 years. However, the aforementioned transition zone of ‘needle like’ structures remained in the majority of corneas. Kymionis et al noted that these hyper-reflective structures demonstrated a gradual relocation in a posterior-to-anterior pattern during sequential examinations over the follow up period.

The hyper-reflectivity of the extra-cellular matrix and presence of large hyper-reflective bands is responsible for the anterior stromal haze observed on slit-lamp biomicroscopy. Some have proposed that the hyper-reflective bands represent activated keratocyte nuclei. A recent ex-vivo study has suggested that these structures are related to transient corneal fibroblast generation (rather than myofibroblast generation which would result in more permanent haze or scarring) and that eyes with abnormal epithelial basement membranes may produce myofibroblasts and therefore develop permanent haze following cross-linking.

Replenishment of the stromal keratocyte population has been noted to start between the second and third month following cross-linking and is complete after six months. Surprisingly, to date there are no published studies providing quantitative analysis of these changes. The current quantitative study reveals that the mean anterior stromal keratocyte density was significantly reduced in the first 6 months returning to normal levels at 12 months. Posterior stromal keratocyte density was unaffected by the treatment. This agrees
with previous studies which generally report the posterior stroma to remain qualitatively unaffected following cross-linking.\(^4, 48, 49\).

9.9.4 Endothelium

The current study showed no significant changes in endothelial density at any post-operative time point. Mazotta et al.\(^12\) similarly showed that corneal endothelial cell density and morphology remain unaltered one year after corneal cross-linking. In a recent study a significant decrease in endothelial density was noted after collagen cross-linking when the cornea fell below 400µm during the treatment.\(^60\) Previous studies have noted that the corneal thickness should be at least 400µm so that the ultraviolet irradiance remains at 0.32 J/cm\(^2\) at the level of the corneal endothelium. This is well below the established damage threshold of 0.65 J/cm\(^2\).\(^42\) Therefore, in practice endothelial damage should not occur if protocols are properly adhered to.

9.10 CONCLUSIONS

Corneal collagen cross-linking is a promising new technique that aims to halt, or at least slow, the progression of keratoconus. In the current study, over a three year period, a total of 39 subjects with progressive keratoconus were recruited into a randomised control trial of corneal collagen cross-linking. At the time of commencement of this project only one RCT on the topic had been commenced, and the anticipated multicentre USA cross-linking trial has now been abandoned. Due to acknowledged limitations in the published literature the author hopes this RCT will substantially add to our evidence base on the topic.
The mean UCVA in treated eyes improved significantly at three, six and twelve months following collagen cross-linking, however, compared to pre-operative measurements, the mean BCVA in treated eyes only improved significantly at the 3 month time-point in this study. Interestingly, despite this documented improvement in UCVA and BSCVA there was no significant change in the mean refractive spherical equivalent at any time point. Thus although this observation confirms refractive stability, refraction per se appears a poor index to assess improved visual function post cross-linking.

In terms of keratometric measures of stability, progression or improvement, the control eyes exhibited significant increase in both maximum and average keratometry values over the twelve months of follow up - indicating further progression of keratoconus. In contrast, in treated eyes the mean maximum keratometry and mean average keratometry values had decreased at six months, twelve months and 24 months post-operatively. Compared to pre-operative values a significant decrease in simulated corneal astigmatism was also observed in treated eyes at 12 months post-operatively.

Only a small percentage of treated eyes were not stabilized or improved by the cross-linking treatment during the study period, with steepening of the keratometric values in only 9.1% of eyes at 12 months post-surgery.

There was no significant change in central corneal thickness (CCT) in control eyes. However, measured CCT and thinnest point pachymetry in the treated eyes was consistently lower than pre-operative values over the entire follow up period. The evidence base and our own observations suggest this may be an artifactual observation attributable to limitations of the Pentacam tomography system in the presence of corneal haze. In this respect, corneal haze based on Pentacam densitometry peaked at 3 months and slowly resolved over the follow up period.
In vivo confocal microscopy revealed significant corneal changes post-treatment. There was complete loss of the sub-basal nerve plexus and loss of anterior stromal keratocytes immediately post-surgery. However, novel quantitative analysis revealed complete regeneration of the sub-basal nerve plexus and keratocyte repopulation by 12 months post-operatively. Hyper-reflective structures formed a clear demarcation line between the anterior treated zone and the posterior stromal untreated zone. Nonetheless, the deep posterior stroma, behind the treatment zone, and the adjacent corneal endothelium remained normal in treated eyes throughout the study period. Clinically, posterior stromal haze formation was noted in five patients (12.8%) in the early post-operative period (1 - 3 months) but this gradually resolved over the follow up period.

In summary: this RCT provides useful additional evidence that collagen cross-linking in keratoconus appears to be a safe and effective intervention to halt, or minimally reverse, the progression of keratoconus in the majority of eyes. This treatment is associated with an acceptably small risk of further visual compromise and in vivo confocal microscopy confirms a return to preoperative values in terms of sub-basal innervation and keratocyte density within twelve months.
REFERENCES


Section V: Conclusions
Chapter 10: Conclusions
10.1 INTRODUCTION

This thesis summarises a series of inter-related projects pertaining to keratoconus in New Zealand that were conducted by the author between the years 2008-2011. This includes a major baseline review of tomography and the associated features of a large New Zealand population of subjects with keratoconus attending a specialist public hospital service. This initial study enabled a classification of the keratoconic tomography which highlighted the variability and asymmetry of the disease (Chapter 4).

The utility, variation and repeatability of the Ocular Response Analyser (ORA), which was a relatively new ophthalmic instrument at the commencement of these studies, was subsequently assessed in relation to measuring the biomechanical properties of the normal cornea (Chapter 5). This study established baseline ORA data in normal eyes prior to extensive utilisation of this technology in the assessment of keratoconus (Chapter 6). The ORA was subsequently used in the longitudinal analysis of biomechanical properties after corneal collagen cross-linking surgery for keratoconus (Chapters 7 and 8).

The large randomised controlled trial (RCT) of collagen cross linking in keratoconus, (Chapters 7 and 8) one of only three International studies known to the author, followed up a large cohort of subjects over 24 months following surgery. This RCT demonstrated the cross-linking technique to be both effective and safe.

The results from each study have already been discussed in detail in the discussion section of the relevant chapters, however, for completeness and ease of reference the major conclusions that can be drawn from these inter-related clinical studies are now concisely summarised in the following pages.
10.2 COMPUTERISED CORNEAL TOMOGRAPHY AND ASSOCIATED FEATURES IN A LARGE NEW ZEALAND KERATOCONIC POPULATION (CHAPTER 4)

A total of 532 eyes of 266 keratoconic subjects in a large hospital-based population were analysed for tomographic features. The data were assessed for associations between keratoconus risk factors and disease phenotype.

**Conclusions:**

a) Maori and Pacific ethnicities are over represented in the New Zealand keratoconic population. Asian, Pacific and Maori populations had significantly higher rates of family history of the disease, compared to the European population.

b) Thirty six subjects (12.4%) reported a family history of keratoconus which corresponds with internationally reported rates.

c) A positive family history of keratoconus was associated with a significantly lower mean power in the 3mm zone and a significantly greater thinnest point pachymetry. Interestingly, disease severity in subjects with a family history was less than those without a family history.

d) There was no significant correlation between keratoconus severity and anterior apical elevation or posterior apical elevation.

e) The most common axial map morphology was the asymmetric bowtie pattern with inferior steepening, followed by round and inferior steepening.

f) The most common anterior elevation map classification was the spur morphology followed in decreasing frequency by island, irregular ridge and regular ridge.

g) Posterior elevation maps were most commonly classified as island morphology followed by, spur, irregular ridge and unclassifiable.
h) The study identified largely asymmetric corneal disease as assessed by current
tomographic classification systems and only 12.5% of subjects had complete
enantiomorphism between their eyes.

10.3 REPEATABILITY AND VARIABILITY OF INTRAOCULAR PRESSURE AND
CORNEAL BIOMECHANICAL PROPERTIES AS MEASURED BY THE
OCULAR RESPONSE ANALYSER (CHAPTER 5)

This study aimed to investigate diurnal and day to day variations in the ORA measures.
One eye of 34 normal subjects was examined for the purposes of the diurnal variation
study. To identify day to day variation one eye of 28 normal subjects was examined.
Diurnal variation measurements were obtained at hourly intervals between the hours of
8:30 am to 5:30 pm and the time at which each subject awoke was recorded. Inter-day
variation measurements were obtained at the same time of day over 5 consecutive days.

Conclusions:

a) There was no significant variation in Goldmann correlated intra-ocular pressure,
   \(IOP_G\), Corneal compensated intra-ocular pressure \(IOP_{CC}\), Corneal Hysteresis
   \(CH\) or Corneal Resistance Factor \(CRF\) throughout the day.

b) The highest \(IOP_{CC}\) and \(IOP_G\) values occurred in the morning, approximately 1-2
   hours after awakening.

c) The lowest \(CH\) and \(CRF\) values occurred in the morning, 1-2 hours after
   awakening.

d) A moderately significant correlation was noted between the diurnal variation of
   \(IOP\) and \(CRF\). The correlation was stronger for \(IOP_G\) than for \(IOP_{CC}\).

e) There was no significant correlation between \(CH\) and either \(IOP_G\) or \(IOP_{CC}\).
f) There was no significant difference in the mean IOP\textsubscript{g}, IOP\textsubscript{CC} and CRF and CH between any of the consecutive daily measurements.

g) The mean CH was significantly higher than the mean CRF for each day.

10.4 CORNEAL HYSTERESIS AND CORNEAL RESISTANCE FACTOR IN KERATOCONUS (CHAPTER 6)

The aim of this study was to elucidate any relationships between Pentacam derived topographic/tomographic parameters and corneal biomechanical parameters obtained from the Ocular Response Analyser in both keratoconic and normal subjects. Forty-six subjects with keratoconus and 66 healthy participants were recruited.

Three tomographic parameters were analysed: 1) maximum simulated keratometry (Dioptres), 2) central corneal thickness (µm), and 3) posterior apical elevation above the computer generated posterior best fit sphere (µm). The ORA measurements obtained were the corneal hysteresis and the corneal resistance factor.

**Conclusions:**

a) Significant differences between the keratoconic and normal populations were identified in relation to mean maximum keratometry, mean apical posterior elevation, corneal hysteresis and corneal resistant factor.

b) Posterior elevation was significantly and positively correlated with maximum keratometry in both the keratoconic and normal groups.

c) Posterior elevation was significantly correlated with CRF in the keratoconic group but not in normal eyes.
d) Posterior corneal elevation correlated significantly with central corneal thickness in keratoconic and normal eyes.

e) Central corneal thickness was significantly correlated with maximum keratometry in the keratoconic group but not in normal eyes.

f) Central corneal thickness was significantly correlated with CH in the normal group but not in the keratoconic group.

g) Central corneal thickness was significantly correlated with CRF in both the keratoconic and normal groups.

10.5 CORNEAL COLLAGEN CROSS-LINKING (CHAPTERS 7-9)

A total of 39 subjects with progressive keratoconus were recruited into a randomised control trial of corneal collagen cross-linking. Analyses included; visual and refractive assessments, Pentacam topographic and tomographic analyses, analysis of corneal biomechanical changes using the Ocular Response Analyser, and assessment of the micro-structural alterations in the cornea using laser scanning in vivo confocal microscopy (IVCM).

Conclusions:

a) Compared to pre-operative measurements the mean UCVA in treated eyes improved significantly at three six and at twelve months following the collagen cross-linking procedure.

b) Compared to pre-operative measurements the mean BCVA in treated eyes only improved significantly at 3 months post-operatively. There was no significant change in BCVA at any time point when compared to baseline in control eyes.
c) There was no significant change in the mean refractive spherical equivalent at any time point when compared to baseline in treated and control eyes.

d) Compared to baseline values there were significant increases in maximum keratometry and average keratometry values over the twelve months of follow up in the control eyes indicating further progression of the untreated eyes.

e) A significant decrease in the mean maximum keratometry and the mean average keratometry values was observed at six months, twelve months and 24 months post-operatively.

f) When compared to pre-operative values a significant decrease in simulated corneal astigmatism in treated eyes was observed at 12 months post-operatively, however, no change was observed in the control eyes.

g) Continued steepening of the keratometric values was noted in 9.1% of the treated eyes at 12 months post-operatively.

h) There was no significant change in central corneal thickness (CCT) in control eyes. CCT and thinnest point pachymetry in the treated eyes was consistently lower than pre-operative values over the entire follow up period. This may be attributed to limitations of the Pentacam system in measuring corneal pachymetry in the presence of corneal haze.

i) Corneal haze (quantified using Pentacam densitometry) peaked at 3 months post-operatively and progressively reduced over the subsequent follow up period.

j) In vivo confocal microscopy revealed significant changes in corneal stromal morphology after the treatment with complete loss of the sub-basal nerve plexus and loss of anterior stromal keratocytes immediately following treatment.

k) Quantitative analysis revealed regeneration of the sub-basal nerve plexus and keratocyte repopulation of the stroma is complete by 12 months post-operatively.

l) There were no alterations in the posterior stroma and corneal endothelium in the treated eyes throughout the study period.
m) Hyper-reflective structures in the corneal stroma were noted to form a clear demarcation line between the anterior treated zone and the posterior untreated zone.

n) Clinically, posterior stromal haze was noted in five patients (12.8%) at 1 - 3 months post-operatively which gradually resolved over the follow up period.
Computerized corneal tomography and associated features in a large New Zealand keratoconic population

Charlotte A. Jordan, BOptom, Azra Zamri, BSc, Catherine Wheldon, MRCoPhth, Dipika V. Patel, PhD, MRCoPhth, Richard Johnson, BOptom, Charles N.J. McGhee, PhD, FRCOphth

Purpose: To evaluate corneal topographic features of keratoconus and associations between risk factors and disease phenotype in New Zealand.

Setting: Departments of Ophthalmology, University of Auckland and Auckland District Health Board, Auckland, New Zealand.

Design: Clinic-based, cross-sectional study.

Methods: The medical records and corneal tomography of patients attending a subspecialty service were reviewed. Data included age, sex, ethnicity, ocular history, family history, atopy, and eye rubbing. Optos II parameters included simulated keratometry, line power, pachymetry, location of maximum power, anterior best-fit sphere (BFS) and posterior BFS. Morphology was categorized by the Rabenstein topography classification.

Results: Final analyses included 532 eyes (266 patients; 144 men) with a mean age of 29.3 years ± 11.56 (SD). Maori and Pacific patients were overrepresented (P = 0.001). Family history of keratoconus was associated with a lower mean corneal power (P = 0.01) and greater pachymetry (P = 0.03). Comparing patients with family history and patients with atopy showed differences in thinnest-point pachymetry (mean: family history, 540 ± 15 μm; atopy 301 ± 6 μm) (P = 0.018). Keratoconus was classified as severe (58.8%) or moderate (33.8%) on mean keratometry. Axial keratometric maps were predominantly asymmetric bow-tie (29%), round (18%), or inferior steepening (17%). Anterior elevation maps were classified as spur (49.3%), island (24%), irregular ridge (15%), or other (11.3%). Eighteen patients (12.5%) had complete excimerization.

Conclusions: Advanced keratoconus was largely asymmetric and differences in tomographic phenotype were associated with differing atopic risk factors. Maori and Pacific ethnicities were overrepresented in this population.

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Keratoconus is a noninflammatory progressive ectasia of the cornea that has a variable reported prevalence of between 50 and 250 per 100,000. An increased prevalence of this disease in the New Zealand population has long been postulated; evidenced by the high national rate of corneal transplantation for keratoconus compared with international data. Reported associations with keratoconus include family history, atopy, and eye rubbing; and in New Zealand the national prevalence of keratoconus may be influenced by high rates of eczema (15%), asthma (22.1%), and systemic allergy (11.4%). The rate of family history of keratoconus has been reported to range from 6% to 20%. and a predominantly autosomal dominant mode of inheritance is presumed; however, many researchers concur that the disease etiology is multifactorial, with genetic and environmental elements coupled with variable expressivity. The diagnosis of keratoconus is made on the basis of a combination of clinical signs and corneal topographic/ tomographic signs. Classic clinical signs of keratoconus include corneal thinning, Fleischer ring,
Vogt's sign and the Wilson sign.  

262 25 Computerized videokeratography is a well-established tool for identifying and monitoring patients with keratoconus.  

263 Lit et al. 26 identified 20 features of videokeratography data and clinical signs to produce a practical classification system for identifying early keratoconus.  

264 The Orbscan II tomographer (Bausch & Lomb) combines Placido disk and slit-beam scanning technologies to analyze and quantify the anterior corneal curvature and enables accurate imaging of the anterior and posterior corneal elevation with wide-field measurement of corneal thickness.  

265 The tomographer has been used to quantitatively document the progression of keratoconus, identifying increased apex elevation, displacement of the apex, and decreased thinnest point pachymetry during progression over a 2-year period.  

266 Several reports have identified topographic parameters important in distinguishing keratoconus from normal corneal morphologic features; these include asymmetric bow-tie astigmatism, posterior corneal elevation, local areas of increased surface power, central corneal keratometric steepening, and inferior-superior dioptric asymmetry.  

267 Keratoconus is largely regarded as a bilateral but asymmetric disease, affecting 1 eye earlier and more severely in most cases. Reports of inter-eye asymmetry between the corneal curvatures of keratoconus have noted differences of 3.50 to 4.00 diopters (D).  

268 Although the causes of this asymmetry have yet to be established, the aim of this study was to evaluate the tomographic features and association between keratoconus risk factors and disease phenotype in a large hospital-based population in New Zealand. The analysis of such factors has not been performed in such a large population of New Zealand keratoconus patients. 

269 The data are particularly interesting in the New Zealand context because New Zealand appears to have a particularly high prevalence of keratoconus. However, because the majority of the New Zealand population are of Northern European ethnicity (67.6%), these data also have relevance to keratoconus globally.

PATIENTS AND METHODS

This study reviewed a large database of keratoconus patients analyzed by Orbscan II tomography attending sub-specialty corneal and external disease clinic of the Department of Ophthalmology, Auckland District Health Board, in conjunction with the Department of Ophthalmology, University of Auckland, New Zealand.

To exclude misdiagnoses, all patients with a diagnosis of keratoconus were identified from the clinical database and the clinical data were reconfirmed in conjunction with a review of all relevant tomography maps by 2 experienced examiners (C.L., R.L.), both licensed optometrists with 5 years and 17 years experience, respectively, based primarily in a public hospital anterior segment service providing contact lens and other keratometric patients.

Diagnostic criteria included tomographic maps with abnormal areas of central, inferior, or superior steepening in conjunction with any of the following: dislike or nonorthogonal astigmatism over 1.50 D, simulated keratometry (K) greater than 47.00 D, or central corneal thickness less than 500 µm, as based on Lim et al. 7 classification scheme for suspect keratoconus.

Having assessed the corneal tomography and made the provisional diagnosis of keratoconus, the clinical notes were accessed for each patient. These included an assessment by a sub-specialist corneal surgeon in relation to the other features of keratoconus, including visual field testing, prominent corneal nerves, Vogt's sign, Munson's sign, Fleischer ring, a subepithelial opacities, and an oil droplet reflex on slit-lamp examination. Data were also collected on age, sex, self-reported ethnicity, and where available, personal and family ocular histories, reported astigmatic condition, and history of eye-rubbing. (Because these patients attended an specialist corneal and external disease clinics, the latter data were usually recorded.)

In all patients, the more severely affected eye was used for analysis based on the maximum K data. Six quantitative Orbscan II tomographic parameters were analyzed: (1) vertex K (maximum and minimum), (2) mean power in the 3.0 mm zone, (3) thinnest pachymetry (defined as pachymetry along the instrument axis, that is, the center of the Orbscan map), (4) location of maximum dioptric power relative to fixation point (including meridian, radius, elevation, and local pachymetry), (5) anterior best-fit sphere (BFS) in millimeters, and (6) posterior BFS in millimeters. The positions of the anterior and the posterior apex were used solely in terms of position in relation to x-y coordinates.
standardized and repeatable measure at a single time point and allows comparison with other published studies assessing elevation using the same system.29

Keratoconic eyes were further classified by severity based on the simulated maximum K value. Severity classifications were based on the Collaborative Longitudinal Evaluation of Keratoconus (CLEK) guidelines, where a maximum simulated keratometric value of less than 45.00 D is classified as mild keratoconus, 45.00 to 52.00 D as moderate keratoconus, and more than 52.00 D as severe keratoconus.29,32 There is no widely accepted severity classification system for keratoconus based on anterior or posterior elevation or wide-field pachymetry maps; therefore, to enable ready comparison with other published studies, the CLEK study topography classification system29,32 was used.

Eyes were also classified by axial map morphology based on the Rabourn classification system (Figure 1).32,33 Axial maps were classified into 1 of 10 subgroups: round, oval, superior steepening, inferior steepening, and irregular symmetrical, symmetrical with skewed axis, asymmetric with inferior steepening, asymmetric with superior steepening, and asymmetric with skewed axis.

Elevation map morphology was classified based on the system reported by Nasalai et al.34 (Figure 2). To determine symmetry or asphericity, only patients with bilateral pachymetry data were used.

The ethnicity statistics for the overall population of patients attending the tertiary ophthalmology facility, within which the cornea and external disease clinics are based, were obtained from the Clinical Data Analyst, Auckland District Health Board.

Any patient whose clinical documents could not be accessed or any patient misdiagnosed based on corneal tomography maps was excluded. Maps were also excluded if the associated clinical features may have created artifacts and made them potentially inaccurate in terms of classification (e.g., corneal scarring, concurrent ocular surface disease, previous surgery, or rigid contact lens wear immediately before tomography acquisition). Orbscan tomographic maps were excluded from analysis if the central 3.0 mm of the scan was not essentially complete (i.e., no significant missing data points). If the scan was too irregular for adequate analysis, any map output did not include all 4 descriptive maps provided by the tomographic system (axial map, pachymetry map, and the anterior and posterior elevation maps), or when obvious artifacts were present (e.g., when very high keratometric values [> 70.00 D] were obtained that were inconsistent with clinical findings).

The study design adhered to the tenets of the Declaration of Helsinki with institutional research ethics board approval.

Statistical Analysis

Multifactorial analysis was performed using SAS software (version 9.1, SAS Institute, Inc.). To assess variables that are associated with individual characteristics of keratoconus, separate general linear models were fitted for the outcomes of severity (maximum K), mean power in the 3.0 mm zone, fixation pachymetry, thinnest pachymetry, and location of maximum diopter power relative to fixation point (including meridian, radius, elevation, and local pachymetry). The x, y coordinates of the position of the thinnest point and the anterior and posterior poles were also used as multivariate outcomes.

Exploratory variables included in all analyses were presence of allergies, a family history of keratoconus, history of eye rubbing, age, sex, and ethnicity. Comparison of axial map classification systems incorporated exploratory factors using 1-way analysis of variance (ANOVA) with the Tukey multiple-comparison post hoc test. Statistical significance was defined as a P value less than 0.05.

RESULTS

Six hundred and one eyes of 353 patients were identified with a diagnosis of keratoconus over a 4-year period. Eighty-seven patients were excluded because of excessive scarring (hydrops), concurrent rigid gas-permeable contact lens, or previous ocular surgery. Therefore, the final analyses included 532 eyes of 266 patients. The mean age of the 122 women (46%) and 144 men (54%) was 29.3 years ± 11.6 (SD) (range 10 to 79 years).

For the purpose of analysis in relation to risk factors and severity, only the more severely affected eye of each patient was selected. Of these 266 patients, 144 (54%) had complete Orbscan II data available for

Figure 1. Classification maps for axial map morphology of the most severely affected eye (n = 266) (after Rabourn et al.33).
both eyes, and these were also included in the analysis of enantiomorphism.

Ethnicity

Table 1 shows the self-reported rates of ethnicity in keratoconic patients compared with the overall population of patients attending the Ophthalmology Department, Auckland District Health Board. The keratoconic group had significantly higher proportions of Pacific and Asian patients and lower rates of Europeans and Asians than the total population (P=0.001). Asian, Pacific, and Māori populations had significantly higher rates of familial history of the disease than the European population (Table 2).

Severity Classification

The majority of the eyes were classified into the severe keratoconus category, followed by the moderate and mild categories (Table 2). The mean thinnest pachymetry differed significantly between the mild (mean 436 ± 69 μm), moderate (mean 414 ± 51.79 μm), and severe (mean 385 ± 65 μm) keratoconus groups (P<0.001, 1-way ANOVA). When analyzing the data overall, significant negative correlations were found between the severity of keratoconus and the thinnest point pachymetry (r = -0.57, P<0.0001), fixation pachymetry (r = -0.465, P<0.0001), and apical pachymetry (r = -0.645, P<0.0001). However, there was no significant correlation between keratoconus severity and anterior apex elevation (r = -0.056, P=0.30) or posterior apex elevation (r = -0.061, P = 30).

No significant association was identified between disease severity and a self-reported history of eye rubbing (P=0.65). This interaction was therefore removed from subsequent analysis.

There was no significant relationship between disease severity and patient age (P=0.50), patient sex (P=0.19), ethnicity (P=0.19), or the presence of concurrent allergies (P=0.43). A family history of keratoconus was the only variable that showed strong evidence of an association with severity of keratoconus (P=0.006), with a positive family history being indicative of a severe disease. When compared with patients without a family history of keratoconus, a positive family history of keratoconus was associated with a significantly lower mean power in the 3.0 mm zone (P=0.01) and a significantly greater thinnest point pachymetry (P=0.01).

Atopy, Age, and Sex

Seventy-four patients (27.8%) had a history of ocular allergy, 76 patients (28.6%) were asthmatic, and 65 patients (24.4%) had eczema. Twelve patients reported a history of ocular allergy, asthma, and eczema (1.5%). Of patients who reported a history of eye rubbing (Table 4), 32 (12.0%) had a sole risk factor of ocular allergy, 19 (7.1%) had the sole risk factor of asthma, and 15 (5.6%) had the sole risk factor of eczema. There was no significant association between age, sex, and ethnicity and the presence of allergy and eye rubbing and the topographic parameters evaluated (Table 4).

Etiological Risk Factors and Tomographic Classification

When comparing statistical outcomes from patients who were reported to have only a family history without other potential risk factors and patients with only
Appendix

Table 1. Distribution of self-reported ethnicity in the keratocono
Table 2. Rates of self-reported family history of keratocono
Table 3. Severity of keratocono based on simulated K, with
Table 4. Rates of self-reported atopy, eye rubbing and family

atopy, there were statistically significant differences
between the mean thinnest pachymetry (family his-
tory 340 ± 15 µm, atopy 381 ± 8 µm; P = 0.018) and
apical pachymetry (family history 335 ± 18 µm, atopy
401 ± 15 µm; P = 0.071). There was also a statistically
significant difference in thinnest pachymetry between
patients with and without a family history of keratocono-

Axial Map Morphology

The most common morphology identified was the
asymmetric bow-tie pattern with inferior steepening,
in 81 eyes (29%). The next most common morphologies
were round 51 (18%) and inferior steepening 46 (17%)
(Figure 1). An interobserver Cronbach's α of 0.966 indi-
cated good classification repeatability between the
observers.

Anterior Elevation Map Morphology

The most common anterior elevation map classifica-
tion was spur morphology (138 [49.3%]). This was
followed in decreasing frequency by island (67
[24.0%]), irregular ridge (42 [15.0%]), regular ridge
18 [6.1%]), ungradable (13 [4.6%]), and incomplete
ridge 2 (0.7%). An interobserver Cronbach's α of 0.999
indicated good classification repeatability between
the observers. Anterior elevation maps were classified
as spur (49.3%), Island (21.0%), irregular ridge (15.0%),
or other (15.5%).

Posterior Elevation Map Morphology

Posterior elevation maps were classified as island
morphology (12 [43.3%]), spur (106 [37.9%]), irregu-
lar ridge (25 [8.5%]), ungradable (25 [8.5%]), regular
ridge (11 [3.7%]), and incomplete ridge 2 (0.7%). An
interobserver Cronbach's α of 0.996 indicated good classi-
fication repeatability between the observers.

Enanthemorphism

In relation to the symmetry of the anterior BFS, pos-
terior BFS, and axial keratometric map morphologies,
of the 144 patients analyzed, 33 (22.9%) had 2 of 3 iden-
tical classifications between the right and left eyes.
Eighteen patients (12.5%) had complete enanthemo-
rhism with 3 of 3 identical classifications.

DISCUSSION

Although many theories exist regarding the biology of
keratocono, none has yet proven conclusive.22 Common-
ly cited etiologic factors include hereditary,
Table 5. Topographic parameters of the differing subgroups based on potential risk factors of family history, eye rubbing, and atopy.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Mean Severity (Max K [D])</th>
<th>Mean Power in 3.0 mm Zone (D)</th>
<th>Thinnest Point Pachymetry (μm)</th>
<th>Apical Elevation (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family history of keratoconus (n = 36)</td>
<td>56.3 ± 6.5</td>
<td>57.39 ± 4.23</td>
<td>39.9 ± 4.28</td>
<td>0.46 ± 0.47</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>.00*</td>
<td>.019*</td>
<td>.025*</td>
<td>.016*</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td></td>
<td></td>
<td>31</td>
</tr>
<tr>
<td>Eyes rubbing (n = 104)</td>
<td>56.19 ± 9.19</td>
<td>51.29 ± 6.19</td>
<td>37.1 ± 2.21</td>
<td>0.31 ± 0.46</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>.05</td>
<td>.74</td>
<td>.03</td>
<td>.06 ± 0.42</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td></td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>Atopy (n = 85)</td>
<td>54.3 ± 7.40</td>
<td>50.17 ± 5.94</td>
<td>37.1 ± 7.32</td>
<td>0.29 ± 0.55</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>43</td>
<td>62</td>
<td>43</td>
<td>48</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td></td>
<td></td>
<td>33</td>
</tr>
</tbody>
</table>

*Statistically significant

systemic and atopic disease, and mechanical trauma due to eye rubbing.25,26 However, the multifactorial nature of keratoconus and the effect of environmental influence on disease phenotype remain to be fully elucidated.

Ammonge et al.27 studied possible etiologic factors in relation to cone morphology, and using a questionnaire-based method, they assessed age at onset, sex, atopic history, and the number of family members with keratoconus. However, they identified no link between any of the potential etiologic factors and the morphologic features. Others have also failed to identify differences in keratoconic histopathology in respect to patient demographics in those having corneal transplantation.28

Inheritance of keratoconus is largely thought to be autosomal dominant with variable expressivity.6,13,14 The current hospital clinic-based study established a familial rate of keratoconus of 12% in this New Zealand population, which generally corresponds with internationally reported rates of 6% to 20%.15,27 Anecdotally, the prevalence of keratoconus in New Zealand is thought to be higher than reported estimates in other countries.22 It has also been postulated that Maori and Pacific populations have a higher incidence of keratoconus than other populations in New Zealand and the current study tends to support this observation.23 Such population differences may suggest varying genetic influences in these ethnic groups, and this may be reflected by the higher rates of family history occurring in the Maori and Pacific subgroup than in the European keratoconic subgroup. However, in terms of ethnicity, a limitation of this study is that the keratoconic group was compared with a large overall population attending an ophthalmology service rather than with an age-matched general population.

This study elucidated phenotypic differences between patients with and those without a family history of keratoconus. There were significant differences in severity of disease, mean K power (3.0 mm zone), and thinnest pachymetry between these groups. Interestingly, disease severity in patients with a family history was less than those without a family history. This may indicate differences in disease processes in different subgroups or an earlier presentation in those with a family history. The CLEK study27 evaluated disease severity in patients with a family history of keratoconus using univariate analysis and noted no significant relationship. However, in this more severely affected population, using multivariate analysis, we were able to minimize the influence of exogenous variables to better establish relationships.

An association between keratoconus and atopy is long established, although whether this is coincidental or a causal relationship is still under debate.25 A higher incidence of eczema has been reported in keratoconic patients,27 and higher rates of atopic disease (eczema, asthma, and hay fever) were noted in keratoconic patients presenting to Moorfields Eye Hospital, London.29 The Dundee University Scottish Keratoconus Study (DUSKS)30 noted self-reported rates of asthma, eczema, and hay fever of 23%, 14%, and 30%, respectively. In the current study, 26.2% of keratoconic patients had asthma, 22.4% had eczema, and 25.5% reported ocular allergy. Although these results are similar to those in the DUSKS study, and notably the majority of the New Zealand European population originate from the United Kingdom, we noted many more patients with eczema in the New Zealand cohort.

In comparison, the North America-based CLEK study31 found significantly higher rates of systemic allergy (52.9%); however, the authors report lower rates of asthma and eczema. Notably, the incidence of
eczema and asthma in the current study were similar to those observed by Owens and Gamble[25] in an earlier New Zealand study of keratoconus, in which they reported that 34% of patients had asthma and 25% had eczema. These data, in conjunction with the present study, may lead us to postulate that the higher rates of keratoconus may be, directly or indirectly, related to the higher rates of atopic disease in New Zealand populations.

In the current study, there was no significant difference in tomographic phenotype between patients with or without ocular allergy. In contrast, Kaya et al.[26] report significant tomographic differences between patients with atopy and those without atopy in several measures, including mean central and thinnest corneal pachymetry, distance of thinnest point to corneal center, anterior and posterior elevation values, and irregularity indices. Although their study suggested phenotypic variations associated with atopic disease, and therefore potential causal disease mechanisms, our study did not identify similar trends. However, the latter may reflect differences in data collection between the study groups because the current study relied on retrospective review of a hospital-based population with relatively advanced disease.

We found significant differences in the tomographic characteristics of patients with an isolated family history of keratoconus and those with a family history but with a history of any ocular allergy. Those included differences in thinnest pachymetry and position of thinnest pachymetry. Therefore, in this study, patients with ocular allergy as their only etiologic risk factor tended to have less severe disease than those with a family history of keratoconus. This must be considered in the context that, overall, patients with a family history of keratoconus (12.4%) had less severe tomographic features of keratoconus than the remainder of the study population. Selection bias may have influenced these results because family members of affected patients may be more likely to seek disease screening at an earlier stage than patients without a family history.

Chronic, low-grade corneal trauma caused by eye rubbing has long been postulated in the pathogenesis of keratoconus.[27,28] It has been reported that after epithelial wounding, increased levels of interleukin-1 result in significant keratocyte apoptosis and subsequent loss of stromal volume.[29] It has also been postulated that intrinsic abnormalities in enzyme function, present in keratocoeic corneas, combined with the trauma of eye rubbing result in apoptosis of keratocytes in the anterior stroma with subsequent loss of stromal homeostasis.[30] In this context, alteration in expression of apoptotic genes, an increase in keratocyte IL-1 receptors, and a decrease in keratocyte density have all been implicated in the pathogenesis of keratoconus.[31]

The cause-and-effect relationship between eye rubbing, atopy, and keratoconus is not clear.[32,33] It has been reported that atopic traits are more common in patients with keratoconus than in general ophthalmic patients. However, it has been suggested that atopy, itch, and eye rubbing are only relevant in the pathogenesis of keratoconus when the highest levels of these factors are present.[34] The cyclical nature of this relationship cannot be ignored, with atopic disease often leading to eye rubbing and therefore the mechanical trauma mentioned earlier. Also we must consider whether eye rubbing is simply the exogenous second-hit factor that pushes a patient with a genetic predisposition to keratoconus from a subclinical into a diseased state.[35,36] Further associations between systemic diseases and both keratoconus and eye rubbing have also been identified; for example, Leber congenital amaurosis is associated with keratoconus and persistent eye rubbing from the oculodigital response.[34,37]

We observed no significant difference between the tomographic maps of patients with self-reported eye rubbing and those without a history of eye rubbing. In view of the retrospective nature of this study, we cannot definitely exclude phenotypic differences that may occur as a result of eye rubbing because this may have been under-reported. Indeed, the current study had a significantly lower proportion of patients with a history of eye rubbing than similar international studies.[23,31] Liu et al.[40] studied the morphology patterns of 46 normal corneas; 71.8% of the anterior corneal elevation maps and 32.6% of posterior elevation maps were classified as island. In terms of axial keratometric--map morphology, the authors note that symmetric bowtie was the more common keratometric morphology. Keratoconus is often asymmetrical in disease severity between a patient's eyes; however, the topographic morphology is thought to be more symmetrical in nature on videokeratography. However, in the current study only 12.5% of keratoconic patients had complete mirror symmetry of tomographic morphology between the eyes. This suggests that the disease is not only asymmetrical in severity but also in morphological pattern within an individual.

Limitations of our study include that the inclusion of patients with data from 1 available eye only may have altered our results because presumably many of those who had contralateral penetrating keratoplasty originally had more severe disease in that eye. The retrospective design of the study also relied on accurate clinical documentation at the patient's earliest clinic examination. Although it is difficult to accurately assess the completeness of the data acquired from the