Copyright Statement

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognize the author's right to be identified as the author of this thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from their thesis.

General copyright and disclaimer

In addition to the above conditions, authors give their consent for the digital copy of their work to be used subject to the conditions specified on the Library Thesis Consent Form and Deposit Licence.
Characterising and Modifying the Keratoconus Disease Process in New Zealand – The Aotearoa Research into Keratoconus (ARK) Project

Akilesh Gokul

A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy in Ophthalmology

The University of Auckland, 2017
Abstract

This thesis contains six inter-related investigations aimed at improving the understanding of the epidemiology, clinical characteristics and natural history of keratoconus in New Zealand (NZ) and to investigated the safety and efficacy of two novel high intensity, high irradiance accelerated corneal collagen cross-linking (A-CXL) protocols. The project was titled the Aotearoa*1 Research into Keratoconus (ARK) Project.

The ARK Study: Part I investigated the epidemiology and basic clinical characteristics of subjects with keratoconus managed by optometrists in NZ. The results of ARK Part I possibly confirm the long held clinical suspicion that keratoconus has an ethnic predisposition for Māori and Pacific Peoples individuals. Furthermore, the results suggest that keratoconus is potentially more severe among subjects of Māori and Pacific Peoples origin.

The ARK Studies: Part IIA, B and C, investigated the phenotypic clinical characteristics and natural history of keratoconus in NZ utilising both prospective and retrospective designs. ARK Part IIA, indicated that keratoconus may have a greater inter-eye disease asymmetry in NZ and that corneal tomographic features/disease severity are correlated with corneal microstructure and biomechanical integrity. ARK Part IIB and C suggest that keratoconus may continue to progress beyond age 30 years and ARK Part IIB characterised the relationship between corneal curvature/power and contact lens corrected visual acuity.

The ARK Study: Part IIIA focused on the repeatability and comparability of corneal curvature/power and pachymetry in three commonly used corneal tomographers in keratoconic eyes and revealed that; corneal curvature/power was most repeatable on the Pentacam HR while pachymetry was most repeatable on the Galilei G2 and the Orbscan II was least repeatable for all parameters, however all measured parameters cannot be used inter-changeably between the three devices. ARK Part IIIB investigated two novel A-CXL protocols utilising continuous and pulsed high irradiance, high intensity ultraviolet-A irradiation and demonstrated that both are safe and effective at slowing or halting the progression of keratoconus 12-months post-operatively.

The results of the inter-related investigations that form the ARK project provide new data on the epidemiology, clinical characteristics and natural history of keratoconus in NZ and the safety and efficacy of two novel A-CXL protocols.

*Aotearoa is the traditional indigenous people’s (Māori) name for New Zealand.*
Dedication

“No one can whistle a symphony. It takes a whole orchestra to play it.” - Halford E. Luccock, this thesis is dedicated to everyone that made its completion possible.

Acknowledgements

The completion of this thesis would not have been possible without the support of a great many people. I wish to specially acknowledge and sincerely thank my supervisory team, who have inspired, challenged and motivated me to aim and achieve as high as I possibly can throughout my PhD adventure. I could not ask for better mentors.

Professor Charles McGhee you have been a pillar of support, being extremely understanding while also knowing exactly how to motivate me when necessary. Your remarkable intelligence, knowledge and experience coupled with seemingly endless enthusiasm make for an extraordinary mentor. Without your guidance in planning and undertaking of all the projects that form the ARK Project, I do not believe I would have completed this thesis. Your meticulous attention to detail in every facet, from gaining ethical approval to critiquing, editing and formatting this thesis has been invaluable. The skills, work ethic and critical thinking skills that you have bestowed onto me in both the clinical and research realms are the strongest foundation on which to build my academic career and for that I am truly grateful. The pleasure of working with you has and always will be, all mine.

Associate Professor Dipika Patel, you are one of the most efficient, hardworking, intelligent and charismatic people I have ever met. Your attention to detail and work ethic are beyond exceptional; you and Charles are truly forces of nature. You contributed profoundly to every aspect of my PhD studies, from guidance with gaining ethical approval and critiquing and editing my thesis to providing moral support and mentoring me with regards to my clinical and research skills. Without you I would be truly lost and this thesis would not have seen the light of day. I will always strive to mimic your incredible skill and I thank you for always providing me with swift and supportive feedback and guiding me to improve my writing and presentation skills. As for Charles, the pleasure of working with you has and always will be, all mine.
Dr Stuti Misra is a passionate and brilliant young scientist, always wearing a smile and offering a helping hand. Stuti was instrumental in setting up all the inter-related studies detailed in this thesis, particularly with regards to study design and gaining ethical approval. I wish to thank you for imparting some of your impeccable skill with the anterior segment instrumentation utilised in the investigations detailed in this thesis.

I am immensely humbled by the out of this world supervision I have been so privileged to receive, I cannot imagine undertaking my PhD studies without it. Charles, Dipika and Stuti never doubted I could accomplish this task and their support never ceased, even in the least impressive hours of the final sprint to the finish. I believe these three individuals have become lifelong friends whom I look forward to facing many other challenges with.

Hans Vellara my colleague, friend and fellow PhD candidate. Most are not lucky enough to have a companion with whom they share their PhD journey thus I am inconceivably appreciative I had you. The friendship and support you provided throughout my PhD studies was profound and not something I would have been able to do without. Thank you my friend.

I would also like to express my thanks to the corneal surgical team who undertook all of the many corneal cross-linking procedures for the study detailed in chapter 9 of this thesis, especially Dr Jay Meyer. In addition to being paramount to the investigation of corneal collagen cross-linking, Jay has been a great source of motivation, insight, support and friendship.

Dr Jina Han and Jeremy Mathan, it goes without saying that your hard work and dedication in assisting me to collect and process data was vital to this PhD’s completion, my sincere thanks to you.

To my employers/friends and colleagues at the private optometry practices I was employed at during my PhD, particularly Grant Watters, Alex and Naomi Bicheno (Mortimer Hirst Eyecare and Eyewear) and Jeremy Wong (Gates Eyewear), I wish to thank you for always providing words of encouragement and always making work enjoyable. Your support in fostering my clinical development and providing me time off to present my research at conferences and when I needed it most to finish this thesis truly go beyond anything I could have asked for. Grant, I also wish to thank you for your support and advice in carrying out the investigation we collaborated on in this thesis (chapter 7) your experience and knowledge really shone through.
Associate Professor Trevor Sherwin I also wish to thank you for the expert advice and support you provided me throughout my PhD, particularly your guidance with gaining ethical approval and administrative academic matters.

Avinesh Pillai, Research Fellow and Statistical Consultant, Department of Statistics, the University of Auckland, I am immensely indebted to you for the many hours of considerate, friendly and incredibly methodical statistical guidance and advice.

Sue Raynel, Hutokshi Chinoy and Maree McInerney, you all played more than a substantial role in my PhD studies. Your administrative, collegial and emotional support was pivotal to the completion of this thesis and for that I sincerely thank you.

I would like to acknowledge the financial support I received from the University of Auckland and the New Zealand Association of Optometrists (NZAO) throughout my PhD studies through their Doctoral and Post Graduate Scholarship’s, respectively. Additionally, I would sincerely like to thank Wilson Sue and Dr Lesley Frederickson for their support and advice throughout my PhD. The NZAO and Cornea and Contact Lens Society of New Zealand were also critical to the success of the ARK project, assisting in spreading the word to optometrists and always supporting the project.

I am eternally indebted to the optometrists that participated in the ARK Study: Part I, without you this study could not have been undertaken. The responses we received from you in this study were phenomenal and prove that our optometrists are truly world-class clinicians. I thank you for being enthusiastic throughout the project, even though it lasted two years.

To my girlfriend Dr Isabella Cheung, you have been beyond supportion, sacrificing many a weekend and evening meal together to allow me to pursue my PhD studies. Your constant words of encouragement and support are something I will always appreciate. I am also grateful for your editing and proof reading of this thesis.

Finally, I would like to thank my family for their emotional support throughout my PhD. I could not ask for more compassionate and understanding parents and brothers; Lusha, Pradeep, Tapan and Prabhat Gokul your unwavering support throughout is very much appreciated.
# Table of Contents

Abstract .............................................................. I
Dedication ............................................................... II
Acknowledgements .................................................... II
Table of Contents ...................................................... V
List of Tables and Figures ........................................... XIII
  List of Tables ........................................................ XIII
  List of Figures ....................................................... XVII
Glossary ............................................................... XXIV
Co-Authorship Forms ............................................... XXVI

Section 1: ................................................................ 1
Overview, Introduction to Keratoconus and Methodology .............. 1
Chapter 1: ................................................................. 2
  Overview of Study Aims and Designs .............................. 2
  1.1 Introduction ....................................................... 3
Chapter 4 – The Epidemiology, Demographics, Distribution and Basic Clinical Characteristics of Subjects with Keratoconus in New Zealand – The Aotearoa Research into Keratoconus (ARK) Study: Part I ............................... 4
Chapter 5 – The Natural History and Severity of Keratoconus in Participants with a Neophyte Diagnosis in the Auckland Region – The Aotearoa Research into Keratoconus (ARK) Study: Part IIA ................................................. 4
Chapter 6 – Assessment of the Natural History of Keratoconus through Retrospective, Longitudinal Analysis of Contact Lens Clinical Records – The Aotearoa Research into Keratoconus (ARK) Study: Part IIB ...................................................... 4
Chapter 7 – The Natural History of Corneal Topographic Progression of Keratoconus after Age 30 years in Non-contact Lens Wearers – The Aotearoa Research into Keratoconus (ARK) Study: Part IIC ............................................................. 5
Chapter 8 – Repeatability and Agreement of Orbscan II, Pentacam HR and Galilei G2Tomography Systems in Corneas with Keratoconus – The Aotearoa Research into Keratoconus (ARK) Study: Part IIIA ........................................ 5
Chapter 9 – Safety and Efficacy of High Intensity, High Irradiance Accelerated Corneal Collagen Cross-linking with Continuous and Pulsed Ultraviolet Exposure – The Aotearoa Research into Keratoconus (ARK) Study: Part IIIB .............................................. 5

Chapter 2: ................................................................. 6
  Introduction to Keratoconus: ....................................... 6
How did we get to where we are? Dr John Nottingham’s 1854 landmark treatise on conical cornea considered in context of current knowledge of keratoconus

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Chapter Overview</td>
<td>7</td>
</tr>
<tr>
<td>2.2</td>
<td>Normal Corneal Anatomy and Physiology</td>
<td>8</td>
</tr>
<tr>
<td>2.2.1</td>
<td>Introduction</td>
<td>8</td>
</tr>
<tr>
<td>2.2.2</td>
<td>Pre-Corneal Tear Film</td>
<td>9</td>
</tr>
<tr>
<td>2.2.3</td>
<td>Corneal Epithelium</td>
<td>9</td>
</tr>
<tr>
<td>2.2.4</td>
<td>Bowman's Layer</td>
<td>10</td>
</tr>
<tr>
<td>2.2.5</td>
<td>Corneal Stroma</td>
<td>10</td>
</tr>
<tr>
<td>2.2.6</td>
<td>Descemet's Membrane</td>
<td>11</td>
</tr>
<tr>
<td>2.2.7</td>
<td>Corneal Endothelium</td>
<td>11</td>
</tr>
<tr>
<td>2.3</td>
<td>Dr John Nottingham’s 1854 landmark treatise on conical cornea considered in context of current knowledge of keratoconus</td>
<td>12</td>
</tr>
<tr>
<td>2.3.1</td>
<td>Introduction</td>
<td>12</td>
</tr>
<tr>
<td>2.3.2</td>
<td>Nottingham: The Surgeon and his Times</td>
<td>14</td>
</tr>
<tr>
<td>2.3.3</td>
<td>Features of Keratoconus</td>
<td>14</td>
</tr>
<tr>
<td>2.3.3.1</td>
<td>Epidemiology and Gender Predisposition</td>
<td>14</td>
</tr>
<tr>
<td>2.3.3.2</td>
<td>Clinical Characteristics</td>
<td>16</td>
</tr>
<tr>
<td>2.3.3.2.1</td>
<td>Slit-Lamp Biomicroscopy Signs</td>
<td>16</td>
</tr>
<tr>
<td>2.3.3.2.2</td>
<td>Corneal Shape</td>
<td>21</td>
</tr>
<tr>
<td>2.3.3.2.3</td>
<td>Corneal Thickness</td>
<td>24</td>
</tr>
<tr>
<td>2.3.3.2.4</td>
<td>Corneal Biomechanics</td>
<td>26</td>
</tr>
<tr>
<td>2.3.3.3</td>
<td>Aetiology and Pathogenesis</td>
<td>27</td>
</tr>
<tr>
<td>2.3.3.3.1</td>
<td>Biochemical and Microstructural Perturbations</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Inflammation</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Nutrition and metabolism</td>
<td>28</td>
</tr>
<tr>
<td>2.3.3.3.2</td>
<td>Microstructural changes in the cornea in keratoconus</td>
<td>29</td>
</tr>
<tr>
<td>2.3.3.3.3</td>
<td>Age of Onset</td>
<td>31</td>
</tr>
<tr>
<td>2.3.3.3.4</td>
<td>Genetics and the Environment</td>
<td>32</td>
</tr>
<tr>
<td>2.3.4</td>
<td>Treatment</td>
<td>33</td>
</tr>
<tr>
<td>2.3.4.1</td>
<td>Optical</td>
<td>34</td>
</tr>
<tr>
<td>2.3.4.1.1</td>
<td>Contact Lenses</td>
<td>35</td>
</tr>
<tr>
<td>2.3.4.2</td>
<td>Medical</td>
<td>36</td>
</tr>
<tr>
<td>2.3.4.3</td>
<td>Surgical</td>
<td>36</td>
</tr>
<tr>
<td>2.3.4.3.1</td>
<td>Refractive Surgery</td>
<td>37</td>
</tr>
<tr>
<td>2.3.4.3.2</td>
<td>Corneal Transplantation</td>
<td>38</td>
</tr>
</tbody>
</table>
2.3.4.4 Mechanical
2.3.4.4.5 Corneal Collagen Cross-linking
2.3.5 Conclusions
2.4 Keratoconus in New Zealand
2.4.1 Epidemiology
2.4.2 Clinical Characteristics of Keratoconus
2.4.3 Conclusions
Chapter 3:
Methodology
Techniques for Assessing Disease Characteristics
3.1 Unaided Vision and Visual Acuity
3.2 Spectacle Refraction
3.3 Slit-lamp Biomicroscopy
3.3.1 Technical aspects of the Slit-lamp Biomicroscope
3.3.2 Utilisation of the Slit-lamp Biomicroscope
3.4 Computerised Corneal Tomography
3.4.1 Orbscan II
3.4.1.1 Technical Aspects of the Orbscan II
3.4.1.2 Utilisation of the Orbscan II
3.4.1 Pentacam HR
3.4.1.1 Technical Aspects of the Pentacam HR
3.4.1.2 Utilisation of the Pentacam HR
3.4.3 Galilei G2
3.4.3.1 Technical Aspects of the Galilei G2
3.4.3.2 Utilisation of the Galilei G2
3.4.4 Medmont E-300
3.4.4.1 Technical Aspects of the Medmont E-300
3.4.4.2 Utilisation of the Medmont E-300
3.4.5 Diagnosis of Keratoconus
3.5 Anterior Segment Optical Coherence Tomography
3.5.1 Heidelberg Spectralis
3.5.1.1 Technical Aspects of the Heidelberg Spectralis Anterior Segment Module
3.5.1.2 Utilisation of the Heidelberg Spectralis Anterior Segment Module
3.6 Corneal Biomechanics
3.6.1 CorVis ST
3.6.1.1 Technical Aspects of the CorVis ST
7.2 Methods 120
  7.2.1 Study Protocol and Design 120
  7.2.2 Outcome Measures 120
  7.2.3 Participants 121
    Inclusion Criteria 121
    Exclusion Criteria 121
    Statistical Analysis 122
7.3 Results 123
7.4 Discussion 128
7.5 Conclusions 131

Section 4: 132
Repeatability and Agreement of Computerised Corneal Tomography Devices and Accelerated Corneal Collagen Cross-linking 132

Chapter 8: 133
Repeatability and Agreement of Orbscan II, Pentacam HR and Galilei G2 Tomography Systems in Corneas with Keratoconus - The Aotearoa Research into Keratoconus (ARK) Study: Part IIIA 133

8.1 Introduction 134
Aims 135

8.2 Methods 135
  8.2.1 Study Protocol and Design 135
  8.2.2 Outcome Measures 136
  8.2.3 Participants 136
    Inclusion Criteria 136
    Exclusion Criteria 136
    Statistical Analysis 137
8.3 Results 138
  8.3.1 Repeatability of Corneal Curvature/Power Measurements 138
  8.3.2 Repeatability of Pachymetry Measurements 138
  8.3.3 Agreement of Corneal Curvature/Power Measurements 139
  8.3.4 Agreement of Pachymetry Measurements 140
  8.3.5 Corneal densitometry measurements 142
8.4 Discussion 142
8.5 Conclusions 145

Chapter 9: 146
Safety and Efficacy of High Intensity, High Irradiance Accelerated Corneal Collagen Cross-linking with Continuous and Pulsed Ultraviolet Exposure - The Aotearoa Research into Keratoconus (ARK)

Study: Part IIIB 146

9.1 Introduction 147

Aims 148

9.2 Methods 148

  9.2.1 Study Protocol and Design 148
  9.2.2 Outcome Measures 149
  9.2.3 Participants 150
    Inclusion Criteria 150
    Exclusion Criteria 151
    Definition of Keratoconus Progression 151
  9.2.4 Surgical Procedure of Corneal Collagen Cross-linking 152

9.3 Results 157

  9.3.1 Clinical complications 157
  9.3.2 Vision and Refraction 158
    9.3.2.1 Vision 158
    9.3.2.2 Refraction 160
  9.3.3 Computerised Corneal Tomography 161
    9.3.3.1 Anterior Power/Curvature 161
    9.3.3.2 Corneal Thickness 163
  9.3.4 Anterior Segment Optical Coherence Tomography 165
    9.3.4.1 Depth of Demarcation 165
  9.3.5 In vivo Confocal Microscopy 166
    9.3.5.1 Corneal Basal Epithelium 166
    9.3.5.2 Corneal Sub-basal Nerve Plexus 167
    9.3.5.3 Keratocytes 168
    9.3.5.4 Corneal Endothelium 169
  9.3.6 Corneal Biomechanics 170
    9.3.6.1 Deformation Amplitude 170
  9.3.7 Treatment Failure 171

9.4 Discussion 171

9.5 Conclusions 176

Section 5: 177

Summary of Aims, Methodology, Results, Discussion and Conclusions 177

Chapter 10: 178
Summary of Aims, Methodologies and Key Results 178

10.1 Introduction: 179

10.2 Aims, Methodology and Key findings of Chapters 4-9 180

10.2.1 Chapter 4 – The Epidemiology, Demographics, Distribution and Basic Clinical Characteristics of Subjects with Keratoconus in New Zealand – The Aotearoa Research into Keratoconus (ARK) Study: Part I 180

10.2.2 Chapter 5 – The Natural History and Severity of Keratoconus in Participants with a Neophyte Diagnosis in the Auckland Region – The Aotearoa Research into Keratoconus (ARK) Study: Part IIA 181

10.2.3 Chapter 6 – Assessment of the Natural History of Keratoconus through Retrospective, Longitudinal Analysis of Contact Lens Clinical Records – The Aotearoa Research into Keratoconus (ARK) Study: Part IIB 182

10.2.4 Chapter 7 – The Natural History of Corneal Topographic Progression of Keratoconus after Age 30 years in Non-contact Lens Wearers – The Aotearoa Research into Keratoconus (ARK) Study: Part IIC 183

10.2.5 Chapter 8 – Repeatability and Agreement of Orbscan II, Pentacam HR and Galilei Tomography Systems in Corneas with Keratoconus – The Aotearoa Research into Keratoconus (ARK) Study: Part IIIA 184

10.2.6 Chapter 9 – Safety and Efficacy of High Intensity, High Irradiance Accelerated Corneal Collagen Cross-linking with Continuous and Pulsed Ultraviolet Exposure – The Aotearoa Research into Keratoconus (ARK) Study: Part IIIB 185

Chapter 11: 186

Discussion and Conclusions 186

11.1 Introduction 187

11.2 Discussion 187

11.3 Conclusions 195

Appendix A – Status of Publications Related to this Thesis 197
Appendix B – Published Articles Related to this Thesis 198
Appendix C – Screenshot of Web-Based Digital Survey for ARK Part I 217
Appendix D – Recruitment Material for ARK Parts I and II: A. Total Vision Care NZAO Newsletter October 2013 and B. CCLS Newsletter January 2014 218
Appendix E – Equipment Survey results NZAO Total Vision Care Newsletter September 2015 – Relevant excerpts of pages 4 and 5 222
References 223
List of Tables and Figures

List of Tables

Table 4-1 Comparison of disease severity between ethnicities using all eyes with available data for $K_{\text{mean}}$, Amsler-Krumeich grade based on $K_{\text{mean}}$ alone, uncorrected vision (UCVA) and best corrected visual acuity (BCVA). Values are mean ± standard deviation or percentage of eyes.

Table 5-1 Descriptive statistics for main outcome measures for the more and less severe eye and difference between the two eyes (n = 60 eyes of 30 participants that underwent a baseline visit); flat simulated keratometry ($K_{\text{flat}}$), steep simulated keratometry ($K_{\text{steep}}$), mean simulated keratometry ($K_{\text{mean}}$), maximum corneal power ($K_{\max}$), thinnest corneal thickness (TCT), central corneal thickness (CCT), unaided vision (UCVA), best spectacle corrected visual acuity (BSCVA), manifest sphere, manifest cylinder, spherical equivalent, sub-basal nerve density, anterior keratocyte density, posterior keratocyte density, deformation amplitude.

Table 5-2 Pearson’s correlation between corneal tomographic parameters; flat simulated keratometry ($K_{\text{flat}}$), steep simulated keratometry ($K_{\text{steep}}$), mean simulated keratometry ($K_{\text{mean}}$), maximum cornea power ($K_{\max}$), thinnest corneal thickness (TCT), central corneal thickness (CCT), and other disease characteristics; unaided vision (UCVA), best spectacle corrected visual acuity (BSCVA), manifest sphere, manifest cylinder, spherical equivalent, sub-basal nerve density, anterior keratocyte density, posterior keratocyte density, deformation amplitude. (n = 60 eyes of 30 participants that underwent a baseline visit)

Table 6-1 Characteristics of the study cohort at their initial presentation: age, gender, follow-up period, contact lens base curve (CLBC), steep keratometry ($K_{\text{steep}}$), flat keratometry ($K_{\text{flat}}$), mean keratometry ($K_{\text{mean}}$) and contact lens corrected visual acuity (CLVA). N = 642 eyes of 406 participants.

Table 6-2 Pearson correlation between contact lens base curve (CLBC), steep keratometry ($K_{\text{steep}}$), flat keratometry ($K_{\text{flat}}$), mean keratometry ($K_{\text{mean}}$) and contact lens corrected visual acuity (CLVA).
Table 6-3 Change in contact lens base curve (CLBC), steep keratometry ($K_{\text{steep}}$), flat keratometry ($K_{\text{flat}}$) and mean keratometry ($K_{\text{mean}}$) between age groups. The p-values represent the result of the repeated measures ANOVA and Bonferroni post-hoc analyses between age groups. Only participants that were present in each of the two age groups being assessed were included in the analysis thus each age group appears twice except for <20 and >60 years. All parameter values are mean ± standard deviation.

Table 6-4 Comparison of mean contact lens corrected visual acuity (CLVA) with increasing corneal curvature/power indicated by groups of incremental increase in curvature/power in contact lens base curve (CLBC), steep keratometry ($K_{\text{steep}}$), flat keratometry ($K_{\text{flat}}$) and mean keratometry ($K_{\text{mean}}$). All parameter values are mean ± standard deviation.

Table 6-5 Comparison of mean contact lens base curve (CLBC), steep keratometry ($K_{\text{steep}}$), flat keratometry ($K_{\text{flat}}$) and mean keratometry ($K_{\text{mean}}$) between eyes with a contact lens corrected visual acuity (CLVA) ≤0.3 and >0.3 LogMAR. All parameter values are mean ± standard deviation.

Table 6-6 Contact lens base curve (CLBC), steep keratometry ($K_{\text{steep}}$), flat keratometry ($K_{\text{flat}}$) and mean keratometry ($K_{\text{mean}}$) as predictors of a contact lens corrected visual acuity (CLVA) >0.3 LogMAR.

Table 7-1 A. Change in computerised topographic parameters between baseline and final reviews in the main study group, 47 eyes of 27 participants; maximum keratometry ($K_{\text{max}}$), steep simulated keratometry ($K_{\text{steep}}$), flat simulated keratometry ($K_{\text{flat}}$), inferior-superior dioptric asymmetry (I-S), surface asymmetry index (SAI) and surface regularity index (SRI). All values are Median (interquartile range) B. Repeatability analysis of $K_{\text{max}}$, $K_{\text{steep}}$, $K_{\text{flat}}$, I-S, SAI and SRI on 37 eyes of 20 randomly selected subjects with keratoconus to determine criteria for progression.

Table 7-2 Spearman correlations and univariate associations for change in computerised corneal topographic parameters, maximum keratometry ($K_{\text{max}}$), steep simulated keratometry ($K_{\text{steep}}$), flat simulated keratometry ($K_{\text{flat}}$), inferior-superior dioptric asymmetry (I-S); between baseline and final review, patient factors, and follow-up time.

Table 7-3 Factors associated with ≥1.00D increase in at least 1 eye in outcome measures with significant change between baseline and final review.
Table 8-1 Intraobserver repeatability for corneal curvature/power and pachymetry measurements obtained with the Pentacam HR, Galilei G2 and Orbscan II; steep simulated keratometry ($K_{steep}$), flat simulated keratometry ($K_{flat}$), central corneal thickness (CCT) and thinnest corneal thickness (TCT). CCT and TCT from Orbscan II assessed without (No AF) and with (0.92 AF) an acoustic factor of 0.92

Table 8-2 Agreement of measurements of corneal curvature/power and pachymetry obtained with the Pentacam HR, Galilei G2 and Orbscan II; steep simulated keratometry ($K_{steep}$), flat simulated keratometry ($K_{flat}$), central corneal thickness (CCT) and thinnest corneal thickness (TCT). CCT and TCT from Orbscan II assessed without (No AF) and with (AF) an acoustic factor of 0.92

Table 9-1 Uncorrected Vision (UCVA) and Best Spectacle Corrected Visual Acuity (BSCVA) pre-operatively at 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation. The p-values in the pre-operative row of the Pulsed group represent the result of independent sample t-test between continuous and pulsed groups at the pre-operative stage. All subsequent p-values pertain to the paired-samples t-test for that variable compared to the pre-operative state. All parameter values are mean ± standard deviation

Table 9-2 Comparison of change in Uncorrected Vision (UCVA) and Best Spectacle Corrected Visual Acuity (BSCVA) pre-operatively and 1, 3, 6 and 12-months post-operatively. All parameter values are the mean difference ± standard deviation of the variable compared to the pre-operative state. P-values pertain to the comparison of the change observed in the continuous and pulsed irradiation groups at the specified time point

Table 9-3 Spherical equivalent refraction pre-operatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation. The p-values in the pre-operative row of the pulsed group represent the result of independent sample t-test between continuous and pulsed groups at the pre-operative stage. All subsequent p-values pertain to the paired-samples t-test for that variable compared to the pre-operative state. All parameter values are mean ± standard deviation

Table 9-4 Comparison of mean change in spherical equivalent refraction pre-operatively and 1, 3, 6 and 12-months post-operatively. All parameter values are the mean difference ± standard deviation of the variable compared to the pre-operative state. P-values pertain to the comparison of the change observed in the continuous and pulsed irradiation groups at the specified time point
Table 9-5 Anterior corneal power/curvature (D), K_{Flat}, K_{Steep} and K_{Max}, pre-operatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation. The p-values in the pre-operative row of the Pulsed group represent the result of independent sample t-test between continuous and pulsed groups at the pre-operative stage. All subsequent p-values pertain to the paired-samples t-test for that variable compared to the pre-operative state. All parameter values are mean ± standard deviation.

Table 9-6 Comparison of change in anterior corneal power/curvature, K_{Flat}, K_{Steep} and K_{Max}, pre-operatively and 1, 3, 6 and 12-months post-operatively. All parameter values are the mean difference ± standard deviation of the variable compared to the pre-operative state. P-values pertain to the comparison of the change observed in the continuous and pulsed irradiation groups at the specified time point.

Table 9-7 Corneal thickness, central corneal thickness (CCT) and thinnest corneal thickness (TCT), in eyes treated with continuous and pulsed ultraviolet-A irradiation. The p-values in the pre-operative row of the Pulsed group represent the result of independent sample t-test between continuous and pulsed groups at the pre-operative stage. All subsequent p-values pertain to the paired-samples t-test for that variable compared to the pre-operative state. All parameter values are mean ± standard deviation.

Table 9-8 Comparison of change corneal thickness, CCT and TCT, pre-operatively and 1, 3, 6 and 12-months post-operatively. All parameter values are the mean difference ± standard deviation of the variable compared to the pre-operative state. P-values pertain to the comparison of the change observed in the continuous and pulsed irradiation groups at the specified time point.

Table 9-9 Depth of demarcation (% corneal thickness) in eyes treated with continuous and pulsed ultraviolet-A irradiation. N indicates the number of participants assessed that had a demarcation line. P-value1 indicates the result of the independent samples t-test comparing the depth of demarcation in the continuous and pulsed groups at different post-operative time points, while P-Value2 indicates the result of the chi square test analysing the difference in the proportion of participants that had a demarcation line in the continuous and pulsed groups.

Table 9-10 Corneal basal epithelial cell density pre-operatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation. P-values pertain to the paired-samples t-test for that variable compared to the pre-operative state. All parameter values are mean ± standard deviation.
Table 9-11 Corneal sub-basal nerve plexus density pre-operatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation. P-values pertain to the paired-samples t-test for that variable compared to the pre-operative state. All parameter values are mean ± standard deviation

Table 9-12 Anterior and posterior keratocyte density pre-operatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation. P-values pertain to the paired-samples t-test for that variable compared to the pre-operative state. All parameter values are mean ± standard deviation

Table 9-13 Corneal endothelial cell density pre-operatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation. P-values pertain to the paired-samples t-test for that variable compared to the pre-operative state. All parameter values are mean ± standard deviation

Table 9-14 Deformation amplitude pre-operatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation. P-values pertain to the paired-samples t-test for that variable compared to the pre-operative state. All parameter values are mean ± standard deviation

Table 9-15 Comparison of change deformation amplitude pre-operatively and 1, 3, 6 and 12-months post-operatively. All parameter values are the mean difference ± standard deviation of the variable compared to the pre-operative state. P-values pertain to the comparison of the change observed in the continuous and pulsed irradiation groups at the specified time point

List of Figures

Figure 2-1 A Haematoxylin and Eosin (H&E) stained cornea through a light microscope indicating corneal epithelium, Bowman’s layer, corneal stroma, Descemet’s membrane and corneal endothelium (Image courtesy of Judy Loh and Associate Professor Trevor Sherwin)

Figure 2-2 Title page of John Nottingham’s treatise; “Practical Observations on Conical Cornea and on the Short Sight and Other Defects of Vision Connected with it”

Figure 2-3 Illustrations of “cornea conica” by Nottingham’s contemporary, Friedrich-August von Ammon, published 1841
**Figure 2-4** The characteristics of keratoconus as seen through the slit-lamp biomicroscope.

A. Optical cross-section of a keratoconic cornea demonstrating increased curvature, anterior chamber depth and areas of thinning

B. Fleischer’s ring (cobalt blue filtered illumination)

C. Vogt’s striae (diffuse illumination)

D. Vogt’s striae (high magnification optical cross-section)

E. Anterior stromal scarring (diffuse illumination)

F. Prominent corneal nerves

**Figure 2-5** The characteristics of keratoconus as seen through the slit-lamp biomicroscope continued.

A. Rizzuti’s sign

B. Munson’s sign

C. Internal reflection observed at the apex in severe keratoconus

D. Active acute corneal hydrops

E. Corneal scarring following the resolution of acute (diffuse illumination)

F. Corneal scarring following the resolution of acute (retro-illumination visualising site of previous break in Descemet’s)

**Figure 2-6** Topographical features of the anterior and posterior cornea in keratoconus. All scans were obtained with the Pentacam HR.

A. Axial power map of an eye with moderate keratoconus demonstrating concentric zones with steep centre and progressive flattening

B. Axial power map of an eye with moderate-advanced keratoconus demonstrating asymmetric vertical steepening (asymmetric bow-tie)

C. Posterior elevation map in an eye with advanced keratoconus

**Figure 2-7** Corneal pachymetry in keratoconus. Corneal thickness map of an eye with moderate keratoconus (obtained with the Pentacam HR)

**Figure 2-8** Wide-field montage of the corneal sub-basal nerve plexus.

A. Normal

B. Moderate keratoconus

**Figure 2-9** Penetrating keratoplasty where the indication was keratoconus through a slit-lamp biomicroscope.

A. A healthy penetrating keratoplasty with interrupted sutures in place – 6 months post-operative

B. A healthy penetrating keratoplasty – 4 years post-operative

**Figure 3-1** Bailey-Lovie vision chart used to measure unaided vision and best corrected visual acuity

**Figure 3-2** The Haag-Streit slit-lamp biomicroscope (Haag-Streit, Koeniz, Switzerland).

A. Haag-Streit slit-lamp biomicroscope with attached Canon digital camera

B. Haag-Streit slit-lamp biomicroscope in clinical use
**Figure 3-3** The Orbscan II (Bausch and Lomb, Rochester, NY, USA)  
A. The Orbscan II with red fixation light and illuminated Placido rings visible  
B. Improper alignment of the Orbscan II, arrows indicate the directions to move the device to obtain proper alignment  
C. Proper alignment of the Orbscan II, arrow highlights the indicators of alignment, red circle centred on Placido ring mires (x and y-axis alignment) and z-axis alignment mires touching to form a “S”  
D. The Orbscan II in clinical use

**Figure 3-4** Standard quad-map of the Orbscan II (Bausch and Lomb, Rochester, NY, USA) in a participant with moderate keratoconus, displaying: axial power, pachymetry, anterior elevation and posterior elevation maps

**Figure 3-5** The Pentacam HR (Oculus, Wetzlar, Germany)  
A. The Pentacam HR  
B. Scheimpflug camera, blue slit beam and static camera  
C. Improper alignment of the Pentacam HR and arrows directing operator to obtain proper x, y and z-axis alignment  
D. Proper alignment of the Pentacam HR  
E. The Pentacam HR in clinical use

**Figure 3-6** Refractive quad-map of the Pentacam HR (Oculus, Wetzlar, Germany) in a participant with moderate keratoconus, displaying: axial power, pachymetry, anterior elevation and posterior elevation maps

**Figure 3-7** The Galilei G2 (Ziemer Ophthalmology Co., Allmendstrasse, Switzerland)  
A. The Galilei G2  
B. Dual Scheimpflug cameras, blue slit beam, static camera and Placido rings  
C. Improper alignment of the Galilei G2, arrows indicate the direction the device is to be moved to obtain proper alignment  
D. Proper alignment of the Galilei G2, arrows highlight the indicators of alignment, red crosshairs with mires (x and y-axis) and anterior edge of Scheimpflug image of the cornea and red horizontal indicator (z-axis)  
E. The Galilei G2 in clinical use

**Figure 3-8** Refractive quad-map of the Galilei G2 (Ziemer Ophthalmology Co., Allmendstrasse, Switzerland) in a participant with moderate keratoconus, displaying: axial power, pachymetry, anterior elevation and posterior elevation maps
**Figure 3-9** The Medmont E-300 (Medmont Pty Ltd, Camberwell, Australia) A. The Medmont E-300 B. Placido rings of the Medmont E-300, arrows indicate the direction the device is to be moved to obtain proper alignment C. Improper alignment of the Medmont E-300, arrows indicate the direction the device is to be moved to obtain proper alignment D. Proper alignment of the Medmont E-300, arrow highlights the indicators of alignment, green cross-hairs centred on centre of Placido ring mires (x and y-axis alignment) and red horizontal line touching horizontal arm of the green cross-hairs (z-axis alignment) and automatic image acquisition

**Figure 3-10** Axial power map of the Medmont E-300 (Medmont Pty Ltd, Camberwell, Australia) in a participant with moderate keratoconus: axial power, simulated keratometry, inferior-superior dioptic asymmetry (I-S index), surface asymmetry index (SAI) and surface regularity index (SRI)

**Figure 3-11** Heidelberg Spectralis Anterior Segment Module (HSAM) (Heidelberg Engineering GmbH, Heidelberg, Germany) A. The HSAM B. Light from the super luminescence diode light source – only visible with a camera sensitive to infrared light C. Improper alignment of the scan area with the central cornea D. Proper alignment of the HSAM E. The HSAM in clinical use

**Figure 3-12** The CorVis ST (Oculus, Wetzlar, Germany): A. The CorVis ST B. Improper alignment of the CorVis ST, the red arrow instructs the operator how to alter the position of the device to obtain proper x, y and z-axis alignment C. Proper alignment of the CorVis ST D. The CorVis ST in clinical use E. Scheimpflug images of the cornea under the influence of the air-pulse emitted from the CorVis ST

**Figure 3-13** The Heidelberg Retina Tomograph II Rostock Corneal Module (HRTII) (Heidelberg Engineering, GmBH, Germany) A. The HRTII B. Application of Viscotears onto objective lens C. Viscotears visible between objective lens and applanating lens cap D. View of lateral CCD camera, focus depth and image of keratocytes being captured E. The HRTII in clinical use

**Figure 3-14** Cell counts or nerve tracings conducted on *in vivo* confocal microscopy images obtained with the Heidelberg Retina Tomograph II Rostock Corneal Module (Heidelberg Engineering, GmBH, Germany) A. Corneal epithelium B. Corneal sub-basal nerve plexus C. Anterior keratocytes D. Posterior keratocytes
Figure 3-15 The Confoscan 4 (Nidek Technologies, Gamagori, Japan) A. The Confoscan 4 B. & C. Application of Viscotears onto the objective lens D. Cell count conducted on *in vivo* confocal microscopy image of the corneal endothelium obtained with the Confoscan 4 E. The Confoscan 4 in clinical use

Figure 3-16 *In vivo* confocal microscopy images of the central cornea obtained with the Confoscan 4 (A, C, E, G and I) and the Heidelberg Retina Tomograph II Rostock Corneal Module (B, D, F, H and J)

Figure 4-1 Comparison of percentage distribution of subjects with keratoconus (N=1869) and overall population across New Zealand, by region. All values are proportion of subjects included in the ARK study vs. proportion of overall population resident in that region

Figure 4-2A. Ethnicity distribution for all cases of keratoconus (N=1869) entered into ARK database B. Ethnicity distribution for all individuals captured in 2013 New Zealand Census

Figure 4-3 A. Types of refractive correction (N=2439 eyes) used to obtain best corrected visual acuity B. Amsler-Krumeich classification (N=2439 eyes) based on keratometry/simulated keratometry alone

Figure 6-1 Comparison of the mean contact lens base curve (CLBC) and contact lens corrected visual acuity (CLVA) between age groups A. Contact lens base curve B. Contact lens corrected visual acuity

Figure 6-2 Comparison of the mean steep keratometry (K_{steep}), flat keratometry (K_{flat}) and mean keratometry (K_{mean}) between age groups A. Steep keratometry B. Flat keratometry C. Mean keratometry

Figure 7-1 Percentage of eyes with no and significant rate of change and magnitude of progression of computerised corneal topographic parameters between baseline and final reviews; maximum keratometry (K_{max}), steep simulated keratometry (K_{steep}), flat simulated keratometry (K_{flat}) and inferior-superior dioptic asymmetry (I-S). A. Rate of Change B. Magnitude of progression between initial and final review.

Figure 7-2 Change in steep keratometry (K_{steep}) between baseline and final review A. 16 eyes of 11 participants with 5 years of follow-up or more B. 27 eyes of 16 participants with less than 5 years of follow-up
**Figure 8-1** Bland-Altman plots showing agreement in steep simulated keratometry ($K_{\text{steep}}$), flat simulated keratometry ($K_{\text{flat}}$), central corneal thickness (CCT) and thinnest corneal thickness (TCT) measurements obtained with the Pentacam HR, Galilei G2 and Orbscan II. Central line represents mean difference between the two devices. Dotted lines represent 95% limits of agreement.

**Figure 9-1** The Avedro KXL (A-KXL) (Avedro Inc., Waltham, MS, USA) A. The A-KXL, arrows indicate the battery housing, central processing unit (CPU) and touch display, positioning arm and ultraviolet-A (UV-A) light source B. A-KXL settings for the continuous treatment group C. A-KXL settings for the pulsed treatment group.

**Figure 9-2** Surgical Procedure of Corneal Collagen Cross-linking A. 8mm optical zone marker with cross-hairs, Tooke’s knife and PVA spear B. Epithelial debridement using the Tooke’s knife C. Takagi Handy Pachymeter P-1 (Takagi Seiko Co. Ltd., Takaoka, Japan) D. Application of riboflavin solution (VibeX Rapid) E. Alignment of red cross-hairs and green alignment dot prior to initialising ultraviolet-A treatment F. Active ultraviolet-A treatment – the appearance of the pulsed treatment is essentially oscillation between E. and F. in 1 second intervals.

**Figure 9-3** Post-accelerated corneal collagen cross-linking anterior corneal stromal haze observed 1-month postoperatively through a slit-lamp biomicroscope A. 16x magnification B. 25x magnification.

**Figure 9-4** Mean Uncorrected Vision (UCVA) and Best Spectacle Corrected Visual Acuity (BSCVA) pre-operatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation.

**Figure 9-5** Mean spherical equivalent refraction pre-operatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation.

**Figure 9-6** Mean anterior corneal power/curvature, $K_{\text{flat}}$, $K_{\text{steep}}$ and $K_{\text{Max}}$, pre-operatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation.

**Figure 9-7** Mean corneal thickness, CCT and TCT, pre-operatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation.

**Figure 9-8** Demarcation line (indicated by the arrows) on anterior segment optical coherence tomography A. Shallow demarcation line B. Deep demarcation line.
Figure 9-9 Corneal sub-basal nerve plexus A. Pre-operative B. Reduced density 1-month post-operatively C. Return to pre-operative density 12-months post-operatively

Figure 9-10 Anterior keratocytes A. Pre-operative B. Hyper-reflective cytoplasm and extracellular lacunae 1-month post-operatively C. Persisting reduction in density 12-months post-operatively

Figure 9-11 Corneal endothelial cells, high density pre-operatively (A.) persisting at 12-months post-operatively (B.)

Figure 9-12 Mean deformation amplitude pre-operatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A
## Glossary

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-CXL</td>
<td>Accelerated corneal collagen cross-linking</td>
</tr>
<tr>
<td>ADHB</td>
<td>Auckland District Health Board</td>
</tr>
<tr>
<td>AG</td>
<td>Akilesh Gokul</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ARK</td>
<td>Aotearoa Research into Keratoconus</td>
</tr>
<tr>
<td>AS-OCT</td>
<td>Anterior segment OCT</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>BCVA</td>
<td>Best corrected visual acuity</td>
</tr>
<tr>
<td>BSCVA</td>
<td>Best spectacle corrected visual acuity</td>
</tr>
<tr>
<td>BSS</td>
<td>Balanced saline solution</td>
</tr>
<tr>
<td>CA-CXL</td>
<td>Continuous high intensity, high irradiance CXL</td>
</tr>
<tr>
<td>CCD</td>
<td>Charge coupled device</td>
</tr>
<tr>
<td>CCLS</td>
<td>Cornea and Contact Lens Society of New Zealand</td>
</tr>
<tr>
<td>CCT</td>
<td>Central corneal thickness</td>
</tr>
<tr>
<td>Cells/mm²</td>
<td>Cells per millimetre squared</td>
</tr>
<tr>
<td>CH</td>
<td>Corneal hysteresis</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CL</td>
<td>Contact Lens</td>
</tr>
<tr>
<td>CLBC</td>
<td>Contact lens base curve</td>
</tr>
<tr>
<td>CLEK</td>
<td>Collaborative Longitudinal Investigation of Keratoconus</td>
</tr>
<tr>
<td>CLVA</td>
<td>Contact lens visual acuity</td>
</tr>
<tr>
<td>CRF</td>
<td>Corneal resistance factor</td>
</tr>
<tr>
<td>CST</td>
<td>CorVis ST</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>CXL</td>
<td>Corneal cross-linking</td>
</tr>
<tr>
<td>D</td>
<td>Dioptres</td>
</tr>
<tr>
<td>DA</td>
<td>Deformation amplitude</td>
</tr>
<tr>
<td>DALK</td>
<td>Deep Anterior Lamella Keratoplasty</td>
</tr>
<tr>
<td>DUSKS</td>
<td>Dundee University Scottish Keratoconus Study</td>
</tr>
<tr>
<td>Fps</td>
<td>Frames per second</td>
</tr>
<tr>
<td>GAG</td>
<td>Glycosaminoglycan</td>
</tr>
<tr>
<td>GLCC</td>
<td>Greenlane Clinical Centre</td>
</tr>
<tr>
<td>GSU</td>
<td>Gray Scale Units</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Haematoxylin and Eosin stain</td>
</tr>
<tr>
<td>HSAM</td>
<td>Heidelberg Spectralis Anterior Segment Module</td>
</tr>
<tr>
<td>IC</td>
<td>Isabella Cheung</td>
</tr>
<tr>
<td>ICC</td>
<td>Intraclass correlation coefficient</td>
</tr>
<tr>
<td>ICRS</td>
<td>Intracorneal ring segments</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IOL</td>
<td>Intra-ocular lens</td>
</tr>
<tr>
<td>I-S ratio</td>
<td>Inferior-superior ratio</td>
</tr>
<tr>
<td>IVCM</td>
<td><em>In vivo</em> confocal microscopy</td>
</tr>
<tr>
<td>J/cm²</td>
<td>Joules per centimetre squared</td>
</tr>
<tr>
<td>K</td>
<td>Keratometry</td>
</tr>
</tbody>
</table>
Co-Authorship Forms

This form is to accompany the submission of any PhD that contains published or unpublished co-authored work. Please include one copy of this form for each co-authored work. Completed forms should be included in all copies of your thesis submitted for examination and library deposit (including digital deposit), following your thesis Acknowledgements. Co-authored works may be included in a thesis if the candidate has written all or the majority of the text and had their contribution confirmed by all co-authors as not less than 65%.

Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.

Chapter 2 - Introduction to Keratoconus: How did we get to where we are? Dr John Nottingham’s 1854 landmark treatise on conical cornea considered in context of current knowledge of keratoconus

Accepted for publication in the journal “Cornea”

Title of submission: Dr John Nottingham’s 1854 Landmark Treatise on Conical Cornea Considered in the Context of the Current Knowledge of Keratoconus

| Nature of contribution by PhD candidate | Study design, review and analysis of Nottingham's treatise and the contemporary literature, preparation of figures, manuscript preparation |
| Extent of contribution by PhD candidate (%) | 85% |

CO-AUTHORS

<table>
<thead>
<tr>
<th>Name</th>
<th>Nature of Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Associate Professor Dipika V Patel</td>
<td>Study design, manuscript critiquing and editing</td>
</tr>
<tr>
<td>Professor Charles NJ McGhee</td>
<td>Study design, manuscript critiquing and editing</td>
</tr>
</tbody>
</table>

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate’s contribution to this work, and the nature of the contribution of each of the co-authors; and
- that the candidate wrote all or the majority of the text.

<table>
<thead>
<tr>
<th>Name</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Associate Professor Dipika V Patel</td>
<td></td>
<td>21/11/16</td>
</tr>
<tr>
<td>Professor Charles NJ McGhee</td>
<td></td>
<td>22/11/16</td>
</tr>
</tbody>
</table>

Last updated: 19 October 2015
Co-Authorship Form

This form is to accompany the submission of any PhD that contains published or unpublished co-authored work. **Please include one copy of this form for each co-authored work.** Completed forms should be included in all copies of your thesis submitted for examination and library deposit (including digital deposit), following your thesis Acknowledgements. Co-authored works may be included in a thesis if the candidate has written all or the majority of the text and had their contribution confirmed by all co-authors as not less than 65%.

Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.

Chapter 4 - The Epidemiology, Demographics, Distribution and Basic Clinical Characteristics of Subjects with Keratoconus in New Zealand – The Aotearoa Research into Keratoconus (ARK) Study: Part I

Submitted for publication in the British Journal of Ophthalmology - currently under peer-review

Title of submission: The Aotearoa Research into Keratoconus (ARK) Study: Part I - Epidemiology, Demographics and Clinical Characteristics of Keratoconus in New Zealand

<table>
<thead>
<tr>
<th>Nature of contribution by PhD candidate</th>
<th>Study design, data collection and processing, statistical analysis of data, preparation of data tables and figures, manuscript preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extent of contribution by PhD candidate (%)</td>
<td>85%</td>
</tr>
</tbody>
</table>

**CO-AUTHORS**

<table>
<thead>
<tr>
<th>Name</th>
<th>Nature of Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jeremy J Mathan</td>
<td>Assistance with data collection and processing</td>
</tr>
<tr>
<td>Jina JY Han</td>
<td>Assistance with data collection and processing</td>
</tr>
<tr>
<td>Dr Stuti L Misra</td>
<td>Study design, manuscript critiquing and editing</td>
</tr>
<tr>
<td>Associate Professor Dipika V Patel</td>
<td>Study design, manuscript critiquing and editing</td>
</tr>
<tr>
<td>Professor Charles NJ McGhee</td>
<td>Study design, manuscript critiquing and editing</td>
</tr>
</tbody>
</table>

**Certification by Co-Authors**

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate’s contribution to this work, and the nature of the contribution of each of the co-authors; and
- the candidate wrote all or the majority of the text.

<table>
<thead>
<tr>
<th>Name</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jeremy J Mathan</td>
<td></td>
<td>22/08/16</td>
</tr>
<tr>
<td>Jina JY Han</td>
<td></td>
<td>2/6/18/16</td>
</tr>
<tr>
<td>Dr Stuti L Misra</td>
<td></td>
<td>22/8/16</td>
</tr>
<tr>
<td>Associate Professor Dipika V Patel</td>
<td></td>
<td>22/1/16</td>
</tr>
<tr>
<td>Professor Charles NJ McGhee</td>
<td></td>
<td>22/8/16</td>
</tr>
</tbody>
</table>

Last updated: 19 October 2015
This form is to accompany the submission of any PhD that contains published or unpublished co-authored work. Please include one copy of this form for each co-authored work. Completed forms should be included in all copies of your thesis submitted for examination and library deposit (including digital deposit), following your thesis Acknowledgements. Co-authored works may be included in a thesis if the candidate has written all or the majority of the text and had their contribution confirmed by all co-authors as not less than 65%.

Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.

Chapter 7 - The Natural History of Corneal Topographic Progression of Keratoconus after Age 30 years in Non-contact Lens Wearers - The Aotearoa Research into Keratoconus (ARK) Study: Part IIC

Submitted for publication in the British Journal of Ophthalmology – currently under peer-review

Title of submission: The Natural History of Corneal Topographic Progression of Keratoconus after Age 30 years in Non-contact Lens Wearers

<table>
<thead>
<tr>
<th>Nature of contribution by PhD candidate</th>
<th>Study design, data collection, statistical analysis of data, preparation of data tables and figures, manuscript preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extent of contribution by PhD candidate (%)</td>
<td>85%</td>
</tr>
</tbody>
</table>

**CO-AUTHORS**

<table>
<thead>
<tr>
<th>Name</th>
<th>Nature of Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grant A Watters</td>
<td>Study design, assistance with data collection, manuscript critiquing and editing</td>
</tr>
<tr>
<td>Associate Professor Dipika V Patel</td>
<td>Study design, manuscript critiquing and editing</td>
</tr>
<tr>
<td>Professor Charles NJ McGhee</td>
<td>Study design, manuscript critiquing and editing</td>
</tr>
</tbody>
</table>

**Certification by Co-Authors**

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate’s contribution to this work, and the nature of the contribution of each of the co-authors; and
- that the candidate wrote all or the majority of the text.

<table>
<thead>
<tr>
<th>Name</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Associate Professor Dipika V Patel</td>
<td>[Signature]</td>
<td>24/8/16</td>
</tr>
<tr>
<td>Grant A Watters</td>
<td>[Signature]</td>
<td>22/08/16</td>
</tr>
<tr>
<td>Professor Charles NJ McGhee</td>
<td>[Signature]</td>
<td>22/8/16</td>
</tr>
</tbody>
</table>

Last updated: 19 October 2015
Co-Authorship Form

This form is to accompany the submission of any PhD that contains published or unpublished co-authored work. Please include one copy of this form for each co-authored work. Completed forms should be included in all copies of your thesis submitted for examination and library deposit (including digital deposit), following your thesis Acknowledgements. Co-authored works may be included in a thesis if the candidate has written all or the majority of the text and had their contribution confirmed by all co-authors as not less than 65%.

Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.

Chapter 8 – Repeatability and Agreement of Orbscan II, Pentacam HR and Galilei Tomography Systems in Corneas with Keratoconus – The Aotearoa Research into Keratoconus (ARK) Study: Part IIIA

Submitted for publication in the American Journal of Ophthalmology – currently under peer-review

Title of submission: Repeatability and Agreement of Orbscan II, Pentacam HR and Galilei Tomography Systems in Corneas with Keratoconus

<table>
<thead>
<tr>
<th>Nature of contribution by PhD candidate</th>
<th>Data acquisition, collection and processing, statistical analysis of data, preparation of data tables and figures, manuscript preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extent of contribution by PhD candidate (%)</td>
<td>70%</td>
</tr>
</tbody>
</table>

### CO-AUTHORS

<table>
<thead>
<tr>
<th>Name</th>
<th>Nature of Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr Jay J Meyer</td>
<td>Study design, manuscript preparation</td>
</tr>
<tr>
<td>Hans R Vellara</td>
<td>Assistance with data acquisition and collection</td>
</tr>
<tr>
<td>Dr Zak Prime</td>
<td>Assistance with data collection</td>
</tr>
<tr>
<td>Professor Charles NJ McGhee</td>
<td>Study design, manuscript critiquing and editing</td>
</tr>
</tbody>
</table>

### Certification by Co-Authors

The undersigned hereby certify that:
- the above statement correctly reflects the nature and extent of the PhD candidate’s contribution to this work, and the nature of the contribution of each of the co-authors; and
- that the candidate wrote all or the majority of the text.

<table>
<thead>
<tr>
<th>Name</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr Jay J Meyer</td>
<td></td>
<td>24/8/16</td>
</tr>
<tr>
<td>Hans R Vellara</td>
<td></td>
<td>21/09/16</td>
</tr>
<tr>
<td>Dr Zak Prime</td>
<td></td>
<td>22/08/16</td>
</tr>
<tr>
<td>Professor Charles NJ McGhee</td>
<td></td>
<td>22/8/16</td>
</tr>
</tbody>
</table>

Last updated: 19 October 2015
Section 1:

Overview, Introduction to Keratoconus and Methodology

Chapter 1-3
Chapter 1:

Overview of Study Aims and Designs
1.1 Introduction
Keratoconus is an ectatic corneal disorder typically characterised by progressive thinning, protrusion, induced irregular astigmatism and visual impairment. The traditional view of the natural history of keratoconus is onset in the teenage years with the condition progressing up until the fourth or fifth decade when it generally arrests. The natural history of keratoconus has been investigated through prospective longitudinal investigations including the landmark Collaborative Longitudinal Evaluation of Keratoconus (CLEK) and Dundee University Scottish Keratoconus Study (DUSKS), however, there have been no direct investigations of the natural history of keratoconus in New Zealand (NZ).

The prevalence estimates of keratoconus vary considerably world-wide, ranging from 6.8–2300 per 100,000. There have been no previous investigations of the prevalence of keratoconus across the whole of NZ. Indeed, the last study of the prevalence of keratoconus was conducted in 1978 and was localised to a single region.

In 2003 Wollensak et al. published a non-randomised clinical pilot study detailing the procedure for corneal collagen crosslinking (CXL) as a means to halt or slow down the progression of keratoconus. Known as the Dresden protocol, the procedure requires removal of the central corneal epithelium, followed by application of riboflavin (vitamin B2) solution and irradiation with ultraviolet-A (UV-A) radiation (370nm). A variety of alterations to the Dresden protocol have been proposed, aimed at increasing the efficiency of the CXL procedure by reducing the time taken to perform the procedure, while maintaining safety and efficacy.

Specific discussion of the current knowledge of keratoconus in NZ and worldwide and its shortcomings is covered in chapter 2. However, it is clear that there is a shortcoming in the understanding of the epidemiology, clinical characteristics and natural history of keratoconus in NZ. Furthermore, the apparently higher prevalence of keratoconus in New Zealand, particularly among individuals of Māori and Pacific Peoples descent, make it an ideal location to investigate the safety and efficacy of accelerated CXL (A-CXL) protocols which have the potential to improve the efficiency of the CXL procedure. Thus the aim of this thesis was to investigate the epidemiology, clinical characteristics and natural history of keratoconus in NZ and to investigate the safety and efficacy of two novel high intensity, high irradiance A-CXL protocols. The project was titled the Aotearoa Research into Keratoconus (ARK) Project.

The purpose of this chapter is to provide a brief overview of the study design and aims of the six chapters in this thesis that contain investigations of keratoconus and form the ARK project.
Chapter 4 – The Epidemiology, Demographics, Distribution and Basic Clinical Characteristics of Subjects with Keratoconus in New Zealand – The Aotearoa Research into Keratoconus (ARK) Study: Part I

The purpose of the ARK Study: Part I was to investigate the epidemiologic, demographic and basic clinical characteristics of individuals with keratoconus managed by optometrists in NZ /Aotearoa. A prospective, longitudinal, nationwide, survey protocol was completed for every patient with keratoconus that underwent a consultation with participating optometrists in a 2-year period.

Chapter 5 – The Natural History and Severity of Keratoconus in Participants with a Neophyte Diagnosis in the Auckland Region – The Aotearoa Research into Keratoconus (ARK) Study: Part IIA

The ARK Study: Part IIA was a prospective longitudinal investigation intended to characterise the corneal phenotypic features of keratoconus at initial diagnosis in NZ and track changes in these features as the disease progressed using advanced anterior segment imaging including corneal tomography, anterior segment OCT, *in vivo* confocal microscopy and corneal biomechanical analysis. Participants with a new diagnosis of keratoconus were to undergo a baseline visit and two follow-up visits 8 months apart.

Chapter 6 – Assessment of the Natural History of Keratoconus through Retrospective, Longitudinal Analysis of Contact Lens Clinical Records – The Aotearoa Research into Keratoconus (ARK) Study: Part IIB

The ARK Study: Part IIB was designed to assess the natural history of corneal curvature/power and CLVA of subjects with keratoconus via retrospective, longitudinal analyses. The contact lens clinical records of two specialist optometry practices were analysed to extract data at yearly intervals concerning corneal curvature/power and contact lens corrected visual acuity.
Chapter 7 – The Natural History of Corneal Topographic Progression of Keratoconus after Age 30 years in Non-contact Lens Wearers – The Aotearoa Research into Keratoconus (ARK) Study: Part IIC

The ARK Study Part IIC aimed to determine if significant progression occurs in older, non-contact lens wearing, subjects with keratoconus and to identify potential predictive factors. Clinical and computerised corneal topography records of subjects with keratoconus attending a specialist optometry practice were retrospectively analysed to identify suitable participants.

Chapter 8 – Repeatability and Agreement of Orbscan II, Pentacam HR and Galilei G2Tomography Systems in Corneas with Keratoconus – The Aotearoa Research into Keratoconus (ARK) Study: Part IIIA

The aims of the ARK Study: Part IIIA were to assess the repeatability and agreement of keratometry and pachymetry measurements obtained using the Orbscan II, Pentacam HR and Galilei G2 tomography systems in corneas with keratoconus.

Chapter 9 – Safety and Efficacy of High Intensity, High Irradiance Accelerated Corneal Collagen Cross-linking with Continuous and Pulsed Ultraviolet Exposure – The Aotearoa Research into Keratoconus (ARK) Study: Part IIIB

The ARK Study: Part IIIB was purposed to investigate the safety and efficacy of two novel protocols for accelerated CXL, which entailed using high intensity, high irradiance UV-A. In one protocol the UV-A was continuous (CA-CXL) and in the other it was pulsed (PA-CXL). The two protocols utilised UV-A with a total irradiance of 7.2 J/cm² and intensity of 30 mW/cm².
Chapter 2:

Introduction to Keratoconus:
How did we get to where we are? Dr John Nottingham's 1854 landmark treatise on conical cornea considered in context of current knowledge of keratoconus
2.1 Chapter Overview

As set out in chapter 1, the focus of this thesis is to investigate the clinical characteristics, natural history and epidemiology of keratoconus in New Zealand and the possible use of two novel protocols of accelerated corneal collagen cross-linking to modify the natural history of keratoconus. The purpose of this chapter is to introduce keratoconus as a disease, providing an overview of the current knowledge of keratoconus and the shortcomings in this knowledge, particularly in NZ, thus creating a foundation from which the subsequent chapters can build. To achieve this goal, this chapter has been divided into three sub-sections, each with a specific purpose. Sub-section 2.2 provides an overview of the anatomy and physiology of the normal human cornea. Section 2.3 provides an overview of the current collective knowledge of keratoconus as whole and the gaps in this knowledge, utilising what is possibly the first accurate description of keratoconus as a frame-work. Finally, section 2.3 explores the current knowledge of keratoconus from a NZ perspective, highlighting the shortcomings in this knowledge pertinent to this thesis.
2.2 Normal Corneal Anatomy and Physiology

2.2.1 Introduction
The dense outer shell of the eyeball is composed of the cornea and the sclera. The cornea is located anteriorly and measures 11 to 12mm horizontally and 9 to 10mm vertically. The convex corneal curvature resembles an ellipse. The central optical zone of the cornea is the most curved with the lowest radius of curvature (7.5 – 8mm) and the curvature reduces toward the periphery. In the healthy population, the mean central corneal thickness measures approximately 500µm with the thickness increasing toward the periphery (approximately 700µm). A sulcus (sulcus sclerae) is observed between the cornea and the sclera where the vascular limbus resides. The corneal-scleral sulcus is the result of a change in curvature between the cornea and the sclera.

The cornea is innervated by the long ciliary nerves derived from the ophthalmic branch of the trigeminal nerve. They follow a radial pattern of penetration into the deep peripheral cornea and track anteriorly to form a sub-basal epithelial nerve plexus. The corneal nerves lose their myelination as they enter the corneal stroma and terminate at the level of the epithelial wing cells. Due to the density of the nerve endings in the cornea, its sensory innervation is several hundred times greater than that of the skin.

The avascular cornea receives the majority of its haematogenous factors required for metabolism and healing from the limbal vascular arcades. These arcades are supplied by the anterior ciliary arteries and also by the vessels derived from the facial branch of the external carotid artery. Glucose and oxygen are paramount to the normal metabolic function of the cornea and these nutrients are delivered primarily through diffusion from the aqueous humour and tear film.

The cornea provides approximately two-thirds of the refractive power of the eye (40-44D). This primarily arises from the difference in refractive index between the atmosphere and the cornea (1.376). Various other aspects contribute towards the corneal optical properties including its transparency, radius of curvature, and smooth anterior surface. These properties are the result of the anatomy and physiology of the layers from which the cornea is composed, including (from anterior to posterior); the pre-corneal tear film, corneal epithelium, Bowman’s layer, corneal stroma, Descemet’s membrane and the corneal endothelium.
2.2.2 Pre-Corneal Tear Film

The pre-corneal tear film is located anterior to and is in contact with the corneal epithelium. The thickness of the pre-corneal tear film varies between 7-50 µm and is composed of two layers; a superficial lipid layer (0.1 µm) and an aqueous-mucin layer (7 µm) composed of both an aqueous and mucin component, the concentration of the mucin component increases in the anterior to posterior direction reaching a maximum at the corneal epithelium. The pre-ocular tear film is pertinent to maintaining corneal epithelial integrity, growth and repair, protecting the cornea from dehydration, and maintaining a smooth optical refracting surface.\textsuperscript{15, 18}

2.2.3 Corneal Epithelium

The corneal epithelium is comprised of non-keratinised, stratified, squamous epithelial cells and is approximately 50 µm thick (Figure 2-1). The corneal epithelium consists of five or six layers of different cell types:\textsuperscript{15}

1. Two or three layers of flat polygonal superficial cells. The surfaces of these cells are covered with microvilli that aid in tear film mucin adhesion. These terminally differentiated superficial cells are eventually sloughed off into the pre-corneal tear film.\textsuperscript{15}

2. Two or three layers of wing cells. These cells represent an intermediate stage of differentiation between the basal and superficial cells.\textsuperscript{15}

3. A single layer of columnar basal cells. Basal cells are the only corneal epithelial cells that undergo mitosis, differentiating into wing cells and eventually superficial cells.\textsuperscript{15}

The process of differentiation takes seven to fourteen days, therefore in combination with the sloughing of the superficial cells; the corneal epithelium is in a constant state of regeneration.

The corneal epithelium contains tight junctions (zona occludens) predominantly between the superficial cell layers and also contains hemidesmosomes and desmosomes within every epithelial layer. These tight junction complexes between adjacent epithelial cells provide an important barrier to external stimuli as they prevent passage of agents into deeper corneal layers. Gap junctions (which allow passage of small molecules) are present in the wing and basal cell layers.\textsuperscript{15}

The basal cell layer of the corneal epithelium is adhered to a basement membrane, which is part of Bowman’s layer, via hemidesmesomes (zona adherens).\textsuperscript{15}
2.2.4 Bowman's Layer

Bowman’s layer is an acellular layer approximately 10µm thick and consists mainly of randomly orientated type I and III collagen (Figure 2-1). These collagen fibres are secreted by the keratocytes located in the corneal stroma. Bowman’s layer is attached by type VII collagen fibrils through to the corneal stroma where they form anchoring plaques with type I collagen. The exact physiological function of Bowman’s layer is still not completely understood but it likely contributes to maintaining corneal epithelial and stromal health.

2.2.5 Corneal Stroma

The corneal stroma constitutes more than 90% of the corneal thickness and is primarily comprised of collagen fibrils with a predominant bias towards type I collagen with smaller amounts of type III, V, and VI (Figure 2-1). These collagen fibrils constitute approximately 70% of the dry weight of the corneal stroma. The diameter of the collagen fibres is 22.5 - 35nm and the distance between them is 41.4 ± 0.5nm. It is thought that the transparency of the cornea is a result of the uniformity of the arrangement of the collagen fibres that make up the corneal stroma, causing destructive interference from scattered incident light rays. The fibres are immersed in proteoglycans and together they constitute the extracellular matrix. Proteoglycans consist of a protein core (lumican, keratocan, mimecin and decorin) and a glycosaminoglycan chain (GAG). The most prevalent GAG in the corneal stroma is Keratan sulphate followed by chondroitin and dermatan sulphates. Proteoglycans play a role in maintaining corneal hydration and modulating collagen fibrillogenesis. Keratocytes are spindle shaped cells that occupy 2-3% of the volume of the corneal stroma. Keratocytes are the main cellular component of the corneal stroma and turn over once every 2-3 years. In response to corneal injury, keratocytes are activated and undergo transformation into myofibroblasts that express smooth muscle actin. Myofibroblasts produce extracellular matrix, matrix-metalloproteinases and cytokines for stromal tissue repair.

Corneal transparency, stability and strength are due to the anatomical and biochemical properties of the stroma. The corneal stroma connects to the anterior sclera at the corneo-scleral limbus. The tissue in the region of the corneo-scleral limbus loses its transparency due to the non-uniform arrangement of the collagen fibrils.

2.2.6 Descemet's Membrane

Descemet’s membrane consists of type IV and VIII collagen, laminin, and fibronectin and is tightly adhered to the posterior surface of the corneal stroma, where it acts as the basement membrane of the corneal endothelium (Figure 2-1).
2.2.7 Corneal Endothelium

The corneal endothelium is composed of a single layer of hexagonal cells that is 5µm thick (Figure 2-1). These endothelial cells are uniform in shape and at birth their density is approximately 4000 cells/mm². The cells that make up the corneal endothelium do not undergo mitosis and therefore decrease in density as one ages. The corneal endothelium is primarily responsible for regulation of corneal hydration via active Na⁺/K⁺ ATPase driven osmotic pump. Loss of function of the corneal endothelium results in an influx of H₂O into the corneal stroma, resulting in swelling (an increase in corneal thickness) and loss of clarity due to the loss of the regular arrangement of the collagen fibres that make up the stroma.

Figure 2-1 A Haematoxylin and Eosin (H&E) stained corneal section through a light microscope indicating corneal epithelium, Bowman’s layer, corneal stroma, Descemet’s membrane and corneal endothelium (Image courtesy of Judy Loh and Associate Professor Trevor Sherwin)
2.3 Dr John Nottingham's 1854 landmark treatise on conical cornea considered in context of current knowledge of keratoconus

2.3.1 Introduction

In 1854, a treatise entitled: “Practical Observations on Conical Cornea and on the Short Sight and Other Defects of Vision Connected with it” was published in London, England, by John Churchill (Figure 2-2). Authored by John Nottingham, this treatise has been widely recognised as the first to accurately describe the disease now known as keratoconus. The treatise itself is 270 pages long, composed solely of text, just over 55,000 words including the preface, and contains no figures or tables. It is written in a style where elegance and extreme detail were opted for over conciseness, as expected for scientific writing of the time. Scientific terms of that time are used throughout, indeed, Nottingham refers to keratoconus under a number of different terms; conical cornea, hyper(k)eratosis, staphyloma, and keratoconus to name a few.

Nottingham’s landmark treatise consists of ideas and observations, made not only by Nottingham himself, but also from authors from England, France, Germany, Holland, Belgium, Italy, Denmark, Sweden, Spain and Portugal. Contributions to the literature by Dr Pickford, Mr Walker, Mr Middlemore, Mr Gervis, Mr W. W. Cooper, Stellwag von Carion, and Cappelletti receive especial mention by Nottingham, while he also acknowledges observations by many other renowned clinicians such as von Ammon and William Mackenzie. References to the contributions in the more historic literature by Burkard David Mauchart, Chevalier John Taylor and Antonio Scarpa are also noted. An account of works by some of these and other authors concerning keratoconus prior to Nottingham in 1854, their context and historic precedence, are elaborated on in a recent review by Grzybowski and McGhee. However, in the current work we focus on the ideas and observations of these authors as recounted by Nottingham.

Nottingham’s treatise is therefore, not merely an account of keratoconus made by a single observer, but a consolidation of the accounts of several observers and their ideas pertaining to the disease, in a structure reminiscent of a modern day review, despite this landmark work being published over 160 years ago. However, the majority of the text is Nottingham’s own observations, ideas, theories, concepts and interpretation of works by the aforementioned authors while approximately 25% of the text is a recount of work by said authors. Additionally, only “in-text” references are made to the authors’ work with no bibliography as such to indicate the publication source of the quoted works.

The technological revolution of the last 30 years has provided sophisticated tools to delve deeper into ocular and specifically corneal disease in a manner that could not have been fathomed by our
predecessors. Since the publication of Nottingham’s treatise, our view of keratoconus, in terms of pathogenesis, diagnosis and treatment, has seen many a paradigm shift. This section of chapter 2 analyzes Nottingham’s treatise in terms of its originality at the time of publication and compares the observations and ideas put forth to the modern scientific literature, thus detailing what is currently known about keratoconus. Along the way brief historical accounts are made of some of the major technological advances and breakthroughs that have resulted in the current understanding of keratoconus.

Figure 2-2 Title page of John Nottingham’s treatise; “Practical Observations on Conical Cornea and on the Short Sight and Other Defects of Vision Connected with it”
2.3.2 Nottingham: The Surgeon and his Times

Born in Yorkshire, UK, in 1810, John Nottingham studied medicine and surgery at Guy’s Hospital, London, UK, and in Paris, France, under Guillaume Dupuytren and Alfred-Armand-Louis-Marie Velpeau, followed by appointment as a house surgeon in 1837 at what was then the Liverpool Infirmary, Liverpool, UK, and undertook general practice in central Liverpool, UK, in 1840. Nottingham co-founded the St. Anne’s Dispensary, Liverpool, England, where he refined his skill in ophthalmology and otorhinolaryngology. He published on subjects in the fields of ophthalmology and otorhinolaryngology. Nottingham was awarded Membership of the Royal College of Surgeons (MRCS) in 1832, Membership of the Royal College of Physicians, London (MRCP) in 1844 and Fellowship of the Royal College of Surgeons (FRCS) in 1846. At the time of publication of his treatise on the conical cornea, Nottingham was a surgeon at the Southern Hospital, Liverpool, where he was appointed in 1850.

John Nottingham was an avid reader and accomplished linguist with a large personal literary and scientific library, which made him an impressive conversationalist. In addition he was a Fellow of the Royal Astronomical Society. Following his retirement he suffered from bilateral cataract until operated on in 1880 and 1881 but later had one eye “extirpated” due to “exposure” and acute inflammation in the winter of 1887. It is reported that he was made invalid in his final 20 years by blindness, bronchitis and later “senile decay”. John Nottingham died age 84 on May 7th 1895.

2.3.3 Features of Keratoconus

2.3.3.1 Epidemiology and Gender Predisposition

In his treatise, Nottingham states; “In some European countries... thousands of cases of ocular disease have occurred, without one sample of this malady being observed... it would seem that those surgeons who are most likely to have paid particular attention to the complaint have often not had one case of it in a thousand, and have seldom exceeded three of it in a thousand”. This indicates that Nottingham believed keratoconus to be fairly uncommon, with an estimated prevalence of below 100, and up to 300 per 100,000. Currently, the prevalence of keratoconus is estimated to range from 6.8–2300 per 100,000. Discrepancies in these estimates of the prevalence of keratoconus may be due to numerous factors, namely; the diagnostic criteria used, the location the study was conducted and the fact that most prevalence studies of keratoconus are case-based.

It is quite possible that keratoconus has an ethnic predilection, indeed, the prevalence of keratoconus appears to be greater in India (2.3%) and Israel (2.34%) than in Denmark (0.086%). A recent study of subjects with keratoconus attending sub-specialty cornea and external disease
clinics in New Zealand (NZ) revealed significantly higher proportions of Māori and Pacific Peoples subjects and lower proportions of subjects of European or Asian origin compared to the total population of patients attending the Ophthalmology service. Trends for corneal transplantation also seem to suggest a particularly high prevalence of keratoconus in NZ when we consider the large variation in transplantation rates for keratoconus by country, as a percentage of total transplantations, reported in the last 20 years: 45.6% and 41.1% (NZ), 31.0% (Australia), 18.6% (Hungary), 13.8% (Canada), 22.5% (United Kingdom), and 16% (United States of America).

Nottingham pondered on ethnic predilection, stating that conical cornea had been observed and treated in the Chinese (whom Nottingham refers to as “Mongolian”) and Caucasian peoples with a preference for the Chinese. In his treatise, Nottingham also quotes a report by Sir John Richardson, stating he “Observed no cases of conical cornea among the inhabitants of the northern regions of America” including among the native “Esquimaux” and “Indians”. In summary, Nottingham stated that in “other varieties of mankind it has yet to be studied” and in the politically unsophisticated language of the era, said it did not occur in “savages”.

In discussing the epidemiology of keratoconus, Nottingham noted the limitation of unclear delineation of the disease when making a diagnosis, observing that many cases of keratoconus would remain undetected as individuals would consider it to be “weakness of the eye” and present to an “optician instead of a surgeon”. In contrast, most modern epidemiological studies of keratoconus occur in tertiary centres such as hospitals, where generally more advanced cases of keratoconus are managed, thus these subjects probably constitute the minority of cases in any community. Indeed, advances in rigid gas permeable (RGP) lens technologies over the past 50 years have made it possible for most cases of mild to moderate keratoconus to be managed by community optometrists.

Keratoconus is well known to affect both genders; Nottingham believed keratoconus to have a female preponderance, stating; “more especially in girls, in whom, between the ages of 10 and 16, it is perhaps most frequently met with”. A recent review by McMonnies addresses the question of gender bias, suggesting an apparent shift over time from a female to a more recent male predominance. He suggests that this apparent shift may be due to the reluctance of males to consult with their eye-care practitioners and respond to surveys in the past. In this review, McMonnies analysed gender in studies published between 1986 and 2009, suggesting a male predominance with 60.2% of males being affected on average in these studies. Both the Collaborative Longitudinal Evaluation of Keratoconus (CLEK) Study and the Dundee University Scottish Keratoconus Study (DUSKS), landmark studies in terms of the natural history of
keratoconus, also show a male predominance: CLEK 55.9%, DUSKS 62.5%. Overall, these data, suggest in contrast to Nottingham’s observations, that there is a male predisposition to keratoconus.

2.3.3.2 Clinical Characteristics

In the preface, Nottingham stated that the anatomy of the cornea in the diseased state was where the published literature had its greatest shortcoming. He attributed this to the rarity of post-mortem corneal assessment, due to the fact that keratoconus does not tend to shorten life.\textsuperscript{26} However, in contrast, McMonnies postulates that there may be fewer older patients with keratoconus being cared for by corneal specialists and specialist contact lens fitters, due to a reduced life expectancy.\textsuperscript{47} A similar hypothesis was also espoused by Pobelle-Frasson et al.\textsuperscript{49} Further investigation is required to confirm or refute this possibility.

In relation to corneal (histo) pathology Nottingham further remarks ‘much that relates to the anatomical state of the cornea may be plainly seen by the observer during life, this, in the main, belongs to the external or anterior surface of the tunic, while the posterior surface, as well as the intervening structure containing the true corneal lamina, are nearly out of the reach of satisfactory observation’.\textsuperscript{26} Importantly, despite the technological limitations of his time, Nottingham was able to observe and deduce a number of the characteristics of keratoconus. Unsurprisingly, with the benefit of advanced diagnostic equipment and our current knowledge of keratoconus, some of his observations and deductions are now known to be incorrect.

2.3.3.2.1 Slit-Lamp Biomicroscopy Signs

The prevailing method of assessing the cornea and anterior segment of the eye, used by Nottingham and other observers in the same period, was by means of the naked eye with or without a simple magnifying lens. While Nottingham’s treatise contains no illustrations, his contemporary Friedrich August von Ammon, whom Nottingham references several times, published a manuscript in 1841 containing illustrations of “cornea conica” (Figure 2-3).\textsuperscript{50} The invention of the slit lamp biomicroscope has been attributed to Allvar Gullstrand, who presented the earliest version of the instrument in 1911, long after the publication of Nottingham’s treatise.\textsuperscript{51} The instrument was produced by the Zeiss factory in Jena and allowed for observation of the anterior segment of the eye, not only under high magnification but also allowed for transparent tissues such as the cornea, to be viewed in cross-section. Hence the anterior and posterior surfaces, as well as the stroma, Bowman’s layer and Descemet’s membrane could be observed in a living person for the first time. The advent of the slit-lamp made possible the observation of many of the key clinical characteristics of keratoconus which are now considered common knowledge but could not be observed in Nottingham’s time.
The incorporation of the slit lamp into clinical practice enabled practitioners to view optical corneal cross-sections and estimate corneal curvature and thickness, as well as anterior chamber depth. Thus it became apparent that corneas affected by keratoconus are thinner, protrude, and have a higher than normal curvature with an increased anterior chamber depth (Figure 2-4A). Fleischer’s ring, a partial or complete iron ring found at the base of the cone was also identified (Figure 2-4B). Histopathology reveals that the ferritin particles that make up Fleischer’s ring are located within and between the cells of the corneal epithelium, especially the basal epithelium. Vogt’s striae are fine parallel lines frequently seen in the posterior corneal stroma on slit-lamp examination of keratoconic corneas (Figure 2-4C. and D.). More recently, in vivo confocal microscopy (IVCM) has revealed that Vogt’s striae may in fact be collagen lamellae under stress as opposed to folds, and striae are also present in the anterior stroma which are not visible on slit lamp examination. Other key corneal signs that are now well-established include anterior stromal scarring (Figure 2-4E.), prominent corneal nerves (Figure 2-4F), Rizzuti’s sign (Figure 2-5A), the bright reflection at the nasal limbus when light is directed at the temporal limbus, and Munson’s sign, a V-shaped deformation of the lower eyelid in down gaze can be observed by both the naked eye and with the slit-lamp (Figure 2-5B).

Of all the aforementioned signs of keratoconus that are visible on slit-lamp examination, Nottingham observed and deduced an astounding number of these considering his complete lack of diagnostic technology in 1854. The signs observed and rationalised by Nottingham included: increased corneal curvature, increased anterior chamber depth, thinning of the cornea and the occasional loss of transparency, which we now know are anterior and/or posterior stromal opacities, as a result of breaks in Bowman’s layer or Descemet’s membrane (corneal hydrops (Figure 2-5D, E and F.)). He also noted that the cornea adopted a “sparkling brilliance” as if “a large and lucid tear is holding on its anterior surface”, the credit for which is given to the “peculiar curve of the cornea and associated luminous reflection and refraction”. This may have been in part a description of what we now call Rizzuti’s sign or the internal reflection observed at the apex in severe keratoconus (Figure 2-5C).

Figure 2-3 Illustrations of “cornea conica” by Nottingham’s contemporary, Friedrich-August von Ammon, published 1841.
Earlier signs of keratoconus that theoretically could have been described in Nottingham’s treatise include the oil drop reflex (Charleaux sign) visible on direct ophthalmoscopy and the scissoring reflex on retinoscopy. However, it was only a few years earlier (1850) that Helmholtz presented the first iteration of the direct ophthalmoscope as the first method of observing the retina in a living person, thus it is unlikely that Nottingham would not have had ready access to this device. As for the retinoscope, Burman first described the principles of retinoscopy in 1861, seven years after Nottingham’s publication.

Thinning of the cornea in keratoconus results in an increased susceptibility to corneal hydrops; ruptures in Descemet’s membrane, leading to an influx of the aqueous humour into the stroma (Figure 2-5D), resulting in oedema which may persist for weeks to months and typically results in scarring (Figure 2-5D, E and F.). Corneal hydrops may be associated with trauma, which can be as mild as a patient rubbing their eyes. Nottingham provides a detailed description of corneal hydrops, which he refers to as “hydrops oculi”, taking into account his own experiences with the condition and those of von Ammon, Dr Bruck and Walther. He noted the loss of transparency and swelling of the cornea, which he correctly attributed to accumulation of the aqueous humour and even alluded to the link with trauma, which he related in a case he observed; “the thin tunic was burst by a slight blow on the eye.”
Figure 2-4 The characteristics of keratoconus as seen through the slit-lamp biomicroscope A. Optical cross-section of a keratoconic cornea demonstrating increased curvature, anterior chamber depth and areas of thinning B. Fleischer’s ring (cobalt blue filtered illumination) C. Vogt’s striae (diffuse illumination) D. Vogt’s striae (high magnification optical cross-section) E. Anterior stromal scarring (diffuse illumination) F. Prominent corneal nerves
Figure 2-5 The characteristics of keratoconus as seen through the slit-lamp biomicroscope continued

A. Rizzuti’s sign  B. Munson’s sign  C. Internal reflection observed at the apex in severe keratoconus  
D. Active acute corneal hydrops  E. Corneal scarring following the resolution of acute (diffuse illumination)  
F. Corneal scarring following the resolution of acute (retro-illumination visualising site of previous break in Descemet’s)
2.3.3.2 Corneal Shape

Protrusion and conical shape are key clinical indicators of keratoconus. The first means of accurately quantifying corneal curvature in the clinical setting was via the keratometer, which Herman von Helmholtz is most often credited with the invention of in the 1860’s, while Thomas Young is acknowledged as establishing the original concept in 1801. However, all the components of the modern keratometer were actually present in the instrument produced by Jesse Ramsden and Everard Home in 1779. Thus this instrument was present long before Nottingham’s treatise, however, it was used by both Young, and Ramsden and Home to measure corneal curvature under the process of accommodation and its clinical application in the measurement of corneal curvature in keratoconus may not have been realised until after Nottingham’s publication.

While keratometry was the gold-standard clinical instrument to measure corneal curvature until relatively recently, measurements are confined to four measurements over the central cornea. Over the last 25 years the keratometer has been superseded by computerised corneal topography and tomography, which enable measurement over much of the cornea through several thousand data points. In addition to wide-field maps of the cornea, computerised corneal topographers produce simulated keratometry (Sim K) values indicating the curvature of the steep and flat meridia thus can produce a measurement of corneal astigmatism, analogous to keratometry.

The original idea on which the first computerised corneal topographers were based was conceived by Antonio Placido, who in 1880, produced a series of concentric alternating black and white rings, now known as the Placido disk, which when reflected by the anterior corneal surface, reveals its contour. In the time-line of our measurement of corneal power and shape, it is important to note that the Placido disk was not conceived or introduced into clinical practice until long after the writing of Nottingham’s treatise. Nonetheless, Nottingham, largely through astute clinical observation, mainly through viewing the eye from the lateral aspect, was able to discern the increase in corneal curvature, central localisation of protrusion and eccentrically located apex associated with keratoconus. Corneal topography has in the last 25 years, expanded our fundamental knowledge of the cornea in keratoconus, and can be considered amongst the most significant advances in the clinical assessment of keratoconus.

A recent review by Piñero et al. characterises the anterior topographical patterns most commonly found in keratoconus, and is briefly summarised here. Corneal topography of keratoconus is characterised by a distinct zone of focal increase in power at the apex (“steepening”), surrounded by concentric zones of progressive reduction in power (“flattening”). The zone of focal steepening usually occupies less than two quadrants and may or may not extend to the limbus (Figure 2-6A).
Generally, the cone apex is located in the inferior mid-periphery (Figure 2-6A and B.). The astigmatism of a keratoconic cornea is typically non-orthogonal and irregular. Similar to regular with-the-rule astigmatism, the vertical meridian tends to be steeper in keratoconus; however, there is hemispherical asymmetry with the inferior hemisphere usually steeper than the superior as in the asymmetric bow-tie pattern (Figure 2-6B.). These features have led to the development of a number of indices that aid in topographic screening for keratoconus, such as the skew of steepest radial axes (SRAX) and inferior-superior dioptric (I-S) index.63

The posterior cornea is also well recognised as developing distinct topographical features in keratoconus. In general the topographic features of the posterior corneal surface are calculated as elevation or depression above a simulated spherical object that best approximates the shape of the posterior corneal surface, called the best-fit sphere (BFS). In keratoconic corneas there is generally marked elevation of the posterior surface above the best fit sphere,64 which similar to the anterior surface topography, is characterised by the greatest elevation above the BFS at the apex and surrounding zones of concentric reduction in elevation (Figure 2-6C.). Interestingly, these changes in the topography of the posterior corneal surface may occur prior to those of the anterior surface.65

Keratoconus is typically a bilateral disease, however, it often presents with marked asymmetry.66 The fellow eye may present with only topographic characteristics but may progress66, 67 from subclinical or “forme fruste” to clinically apparent keratoconus.68 Nottingham makes observations in this area in relation to a case of bilateral, yet asymmetric disease, stating; “To the right eye the above description also applies, but in this eye the conicity, which corresponds as to position, appears to be somewhat less than in the other”.1 Nonetheless, he makes no explicit mention of the progression in the fellow eye.26
Figure 2-6 Topographical features of the anterior and posterior cornea in keratoconus. All scans were obtained with the Pentacam HR

A. Axial power map of an eye with moderate keratoconus demonstrating concentric zones with steep centre and progressive flattening

B. Axial power map of an eye with moderate-advanced keratoconus demonstrating asymmetric vertical steepening (asymmetric bow-tie)

C. Posterior elevation map in an eye with advanced keratoconus
Thinning and protrusion of the cornea in keratoconus results in increased higher order aberrations (HOA). Interestingly, although clinical measurement of HOA is a relatively recent phenomenon, the first aberroscope used to measure optical aberrations in a living subject was actually produced by Marius Tscherning in 1894, where a +4.00 dioptre lens was used to project a grid pattern on its surface onto a subject’s retina, the subject was then requested to draw the pattern and deviations in the sketched pattern relative to the projected pattern provided a semi-quantitative analysis. The first objective method of measuring higher order aberrations, was achieved by means of the modern Hartmann-Shack sensor, designed by Hartmann in 1900 and refined by Shack in 1971, albeit with a similar optical setup to Tscherning’s original design. This latter technology was only widely introduced to the clinical setting in the 21st century and therefore far out of reach of John Nottingham. However, he does report multiple cases of monocular polyopia with no chromatic aberration, which he attributes to the “altered form of the anterior part of the eye-ball”. In a case which he observed of a young man suffering from conical cornea he reports; “Mere double vision is not complained of, but a candle, when looked at, appears like a number of lights, confusedly running into one another; but without coloured fringes, or other phenomena of chromatic aberration”. Nottingham also makes multiple remarks suggesting that spherical aberration plays a role in keratoconus. Thus, Nottingham described the subjective visual phenomena we attribute to the irregular shape and higher order aberrations characteristic of keratoconus, including, higher levels of vertical coma, coma-like root mean square (RMS), trefoil and spherical aberration.

2.3.3.2.3 Corneal Thickness

Nottingham’s treatise provides no consensus in respect to corneal thickness in keratoconus as highlighted in the contemporary literature. He proclaims that; “With regard to the thickness of the cornea in the conical part, some writers have reported it to be increased, others have seen it diminished, and in one instance, recently published, the thickness of the cornea was found equal, and unaltered”. However, Nottingham himself correctly suggested that the central cornea reduces in thickness; “Upon the whole, it would seem that the thinning of the central part of the cornea is the pathological condition most uniformly observed, the state of the exterior rim of this tunic having been different in different cases; normal in thickness, abnormally thick, or abnormally thin”. He bases this opinion on the post mortem examinations performed by Jaeger, Walker and Middlemore, all of whom found the cornea to be abnormally thin in the affected part but varied in their findings in the periphery. Furthermore, he reasoned that a thinned cornea centrally would distend due to the pressure of the aqueous humour, resulting in the observed conical shape. Nottingham then goes on to state that a process of thickening or hypertrophy leading to a conical protrusion of the cornea is less easily understood, instead favouring the thinning or atrophy hypothesis.
The only other method for measuring corneal thickness noted by Nottingham was during the procedure of anterior chamber paracentesis. He presents a report by Mr Gervis who claims to have judged the cornea to be thickened as he passed the point of a needle through the affected part while performing an anterior chamber paracentesis. This method is of course highly subjective, inaccurate and cannot be relied upon. In addition Nottingham states the importance of post mortem examinations but also noted their limitations and emphasises the need for in vivo measurements of corneal thickness; “inasmuch as the precise form of the living eye depends, in a great measure, on its vital conditions; so that the figure of this living and beautiful optical instrument is but imperfectly transferred to the organ which we examine post-mortem. In the study of conical cornea, we should advance considerably if any means of accurately determining the thickness of the living cornea”.26

The first method for the in vivo measurement of corneal thickness is credited to Magnus Blix, based on the specular reflection from the epithelium and endothelium, as described in his 1880 thesis/dissertation titled “Oftalmometriska studier”.14, 73 published a quarter of a century after Nottingham’s treatise, once again this technology was far out of Nottingham and other authors of his time’s reach. Clinically viable versions of this optical pachymeter were available in the mid-20th century such as the slit-lamp attachment produced by Haag-Streit,14 followed by those based on ultrasonography, produced by Fredrick Kremer in 1980.74 More recently, optical means of determining corneal thickness have been incorporated into computerised corneal tomographers (Figure 2-7) and anterior segment optical coherence tomography – these techniques have revealed much about the thickness profile of the cornea in keratoconus.75-77 It is now believed that keratoconus is a thinning disorder affecting the entire cornea, but it is characterised by the greatest amount of thinning at the apex of the ectatic region, with attenuation of thinning toward the periphery.78-80
2.3.3.2.4 Corneal Biomechanics

As previously noted, Nottingham correctly deduced that thinning of the cornea would lead to a conical shape. We now know that the thinning of the cornea in keratoconus is related to loss of the structural integrity of the stromal collagen network that normally maintains corneal shape, although, the exact mechanism by which structural integrity is lost is incompletely understood.\textsuperscript{6, 62} Only relatively recently have methods been developed to measure parameters that may reflect corneal biomechanical properties in vivo, including instruments such as: the Ocular Response Analyzer (ORA, Reichert Inc., Depew, NY, USA) and the CorVis ST (Oculus, Wetzlar, Germany).

The ORA\textsuperscript{81} produces two indices, corneal hysteresis (CH) and corneal resistance factor (CRF), relating to the inherent viscous damping and elastic resistance properties of the cornea, respectively.\textsuperscript{82, 83} However, although these measures may reflect biomechanical aspects of the cornea, they are not direct measures of established mechanical properties such as Young’s modulus of elasticity. Compared to the normal population, CH and CRF have been reported to be significantly reduced in established keratoconus.\textsuperscript{82, 84-86} The CorVis ST on the other hand does measure true physical parameters such as the deformation amplitude (DA) of the cornea under the influence of an air-pulse, though once again this is not a traditional measure of biomechanical properties. Nonetheless, the DA has been demonstrated to be significantly increased in subjects with keratoconus, suggesting that the biomechanical compromise in keratoconic corneas can be measured in vivo though there was significant overlap with the normal population.\textsuperscript{87}
Nottingham and other practitioners of his era were limited to palpation through closed lids or directly on the cornea, nonetheless he does allude to the cornea being less rigid in keratoconus.26 There were two prevailing theories at the time pertaining to the biomechanical state of the cornea in keratoconus; first, “The cornea is weakened and thinned, so that the aqueous humour encroaches upon it, and presses it forward”, second, “The cornea is strengthened and thickened, in such manner that it might even be supposed to encroach on the aqueous humour, and press it backward”.26 As previously mentioned, Nottingham came down on the side of a thinned and weakened cornea, often stating that the cornea was “softened” or “weakened” in recounts of cases he observed.26 Additionally he reports works by prolific authors such as Home and Ramsden, who were of similar opinion.26 Therefore, Nottingham’s correct conclusion that the cornea is thinned and weakened in keratoconus was a monumental step forward, considering that we know now that alteration of corneal biomechanical structure is a defining characteristic of keratoconus. Contemporary tools for measuring corneal biomechanical properties hold promise in terms of diagnosing and grading the severity of keratoconus.82,86

2.3.3.3 Aetiology and Pathogenesis

Keratoconus remains an enigmatic disease in many ways despite the enormous diagnostic and technological advances over the last 160 years. Currently, keratoconus is thought to be multifactorial in nature, being influenced by both genetic and environmental factors, essentially summarised by a “two-hit” hypothesis where an underlying genetic predisposition is exacerbated by environmental factors.88 Additionally it is believed that keratoconus may be a general phenotype that represents the common outcome of a number of different pathways.88 Based on a large number of reviewed cases, Nottingham stated that there were at least two different “modes of origin” to keratoconus and described them as follows; cases of conical cornea where inflammation had not previously existed, and conical cornea after inflammation (whether acute or chronic, severe or barely discernible).26 Thus Nottingham may have recognised that keratoconus may be the end result of different underlying mechanisms, in direct connection with this he noted that keratoconus presented at ages that were too varied and circumstances so different that it would be unlikely that there was one common cause.26 Indeed, although Nottingham described or alluded to many of the clinical features of keratoconus we now know are defining characteristics of the disease, his theories of the aetiology of keratoconus and nature of many of the case reports included in his treatise beg the obvious question; was Nottingham always describing keratoconus? In the context of our current knowledge of corneal disease, since other causes of thinning, scarring and protrusion would have been difficult to discriminate with the limited diagnostic equipment available at that time, almost certainly Nottingham and his contemporaries included some non-keratoconus cases in their analyses.
2.3.3.1 Biochemical and Microstructural Perturbations

Inflammation

John Nottingham presents a great number of cases of supposed keratoconus, observed by himself and others such as Taylor, Scarpa, Mackenzie and von Ammon.\textsuperscript{26} Among these cases, many are associated with inflammation, caused by a multitude of conditions ranging from trauma to smallpox and including iritis and keratitis.\textsuperscript{26}

Traditionally, keratoconus is believed to be a non-inflammatory condition, though recent biochemical discoveries, including elevated levels of inflammatory mediators, such as tumour necrosis factor alpha and multiple interleukin (IL) molecules including IL-6 and IL-17,\textsuperscript{89,90} challenge this assumption. Nonetheless, despite the implication of low-level inflammation in the pathogenesis of keratoconus, the cardinal clinical signs of inflammation are typically absent with the exception of acute corneal hydrops.

In Nottingham’s treatise, as illustrated by several case reports, inflammation in the traditional sense is explicit as a second common association in the aetiology of keratoconus.\textsuperscript{26} Thus it is likely that Nottingham was not always describing cases of keratoconus. It is possible that he was illustrating cases of inflammatory corneal thinning disorders though it is difficult to speculate from his descriptions, considering his technological limitations. Indeed, he may have been discussing a multitude of inflammatory conditions, not all being thinning disorders.

Nutrition and metabolism

Nottingham also speaks of alterations in the nutrition of the cornea and numerous other structures of the eye as being responsible for conical cornea. Of particular note are his statements with regard to the two different “modes of origin”; “The first class of cases may be spoken of as instances of conical cornea from alteration in the nutrition of this membrane without visible traces of inflammatory action” and “the second class, as cases of conical cornea, also from perverted nutrition, but preceded or accompanied, or both, by the visible phenomena of inflammation”.\textsuperscript{26} He uses similar terms numerous times, though never stating specifically what he meant. It is difficult to deduce what Nottingham intended to convey, he may have meant there were literally alterations in the nutrients required for normal corneal function or their supply, or it may have been a more complex relationship, relating to metabolic or cellular function.

Today we understand that keratoconus may be the result of alterations to a number of corneal homeostatic processes. Studies of the histopathology\textsuperscript{91,92} and gene expression\textsuperscript{93} in keratoconus have suggested apoptosis of corneal stromal keratocytes to play a role in its pathogenesis. This has
been further substantiated by *in vivo* confocal microscopy.\(^{94-96}\) It has also been suggested that micro-trauma, eye rubbing and contact lens wear, through a complex relationship between IL-1, the Fas ligand and receptor and keratoconic keratocytes, may set up a cycle of corneal stromal keratocyte apoptosis, contributing to the keratoconus disease process.\(^{88, 97-100}\)

Electron microscopy studies suggest that as keratoconus progresses, collagen fibril diameter reduces, whereas proteoglycan content increases.\(^{101}\) Enzymatic degradation of stromal collagen may be a potential mechanism of corneal thinning in keratoconus. Immunohistochemistry analysis of keratoconic corneal buttons and proteomic tear analysis of keratoconic subjects revealed elevated levels of sub-group of proteases known as matrix metalloproteinases (MMPs) and cathepsins, including MMP-1 and -13, known collagenases.\(^{102, 103}\) MMP-13 levels in the tear film have also been shown to be positively correlated with disease severity\(^{104}\) and may potentially be increased by contact lens wear\(^{105}\) and eye rubbing.\(^{106}\) Tissue inhibitors of matrix metalloproteinases (TIMPs) are downregulated in early keratoconus and upregulated as the disease progresses,\(^{107}\) and TIMP-MMP imbalance is implicated in keratocyte apoptosis in keratoconus.\(^{108}\)

The cornea is susceptible to ultra-violet light damage and oxidative stress, which has been postulated as possible contributing mechanisms in keratoconus. Compared to normal corneas, the cornea of subjects with keratoconus have been shown to have increased levels of free radicals or their markers such as superoxide, generated by stromal fibroblasts,\(^{109}\) nitrite and nitrotyrosine which are markers of peroxynitrite,\(^{110, 111}\) and markers of lipid peroxidation such as 4-hydroxy-2-nonenal and malondialdehyde.\(^{110, 111}\) Additionally, compared with non-keratoconic corneas, keratoconic corneas appear to have reduced levels of anti-oxidative enzymes and molecules such as, superoxide dismutase\(^{112}\) and glutathione,\(^{110}\) further implicating oxidative stress in the pathogenesis of keratoconus. Indeed, antioxidants such as vitamins E\(^{113}\) and B2\(^{114}\) have been shown to be effective in protecting against corneal damage.

Although Nottingham and his contemporaries had a basic understanding of corneal physiology and limited access to anatomical and pathological cornea specimens, he could not have construed the pathological mechanisms that have been elaborated by the advanced techniques of the last 50 years.

### 2.3.3.3.2 Microstructural changes in the cornea in keratoconus

*In vivo* confocal microscopy (IVCM) has been used to reveal many of the microstructural changes that occur in corneas affected by keratoconus. The majority of studies indicate a reduction in cell density of all three major cell types of the cornea i.e. epithelial,\(^{94-96, 115, 116}\) keratocyte\(^{95, 96, 115, 117}\) and endothelial,\(^{95, 96}\) compared to controls.
In terms of corneal anatomy and function Nottingham proposed that anomalies in the nervous system of the cornea may contribute to the aberrant nutrition and hence produce conical cornea, noting: “the manner in which the nutrition of the cornea may fairly be supposed to be perverted, - in connection with which the thought suggests itself, that some altered condition of innervation might here be taken into account.”. He subsequently allocates a reasonable portion of the treatise to this possibility. Herein he describes the observations made by Arlt, where the trigeminal nerve is severed just anterior to the gasserian or trigeminal ganglion in dogs. Arlt describes pupil constriction, which would be due to severing the sympathetic pupillary fibres, as well as loss of sensitivity, conjunctival hyperaemia and eventual corneal opacification. This appears to be a description of exposure, although opacification occurred after just eight to ten days. The results of these experiments were similar to those performed by Danish Physiologist, Hannover, although his experiments consisted of the removal of the superior cervical ganglion in cats.

Nottingham made a very weak connection between keratoconus and altered corneal nerve supply. He presented just two cases, which he believed to be the result of apparent impaired innervation. In the first case, conical cornea is anecdotally linked to a “scrofulous affection” of the neck and neighbouring parts. Nottingham postulated that this affection disturbs the cervical ganglion resulting in conical cornea. The second case is closer in description to a case of corneal exposure. A 7 year old girl who was believed to have lost corneal sensation and as a result ulceration and opacification of the cornea ensues due loss of protection of the “winking motion of the eye-lids” and damage by the “atmosphere” and “detained tears”. Nottingham also postulated altered innervation of the face and dental arches as a cause of keratoconus. He presented a number of cases where conical cornea is linked to tooth decay and tooth removal. Currently there is no compelling evidence of dental disease in the aetiology of keratoconus.

The microstructure of the corneal sub-basal nerve plexus is known to be affected in keratoconus; however, it is still unclear if alterations to its morphology are a primary causative factor or a secondary structural change. Sub-basal nerve bundles have been demonstrated to be increased in thickness but reduced in density, in keratoconic corneas compared to controls. Patel et al. revealed that the reduction in sub-basal nerve density in keratoconus is correlated with a reduction in central corneal sensitivity threshold. Additionally, the use of a IVCM montage technique to produce confluent maps of the corneal sub-basal nerve plexus reveal that the normal pattern of radiating spokes converging to a whorl-like pattern centred 1-2mm below the corneal apex with the greatest nerve density in the region of the whorl-like pattern(Figure 2-8A.), is lost in keratoconus, where the radial spoke organisation of nerve bundles is lost and the central inferiorly displaced whorl-like pattern is instead a tortuous network with many bundles forming closed loops.
The distribution of the nerve bundles also appear to conform to the base of the cone in a concentric pattern.\textsuperscript{118}

Figure 2-8 Wide-field montage of the corneal sub-basal nerve plexus A. Normal\textsuperscript{119} B. Moderate keratoconus\textsuperscript{118}

The arrangement of collagen lamellae in the corneal stroma imparts mechanical strength to the cornea and X-ray diffraction has revealed that in a normal cornea, collagen fibrils in the central cornea take up a preferred orientation, running for the most part, orthogonally in either the superior-inferior or temporal-nasal direction.\textsuperscript{120-122} In keratoconus there are alterations in the distribution and orientation of collagen fibrils, implicating lamellar slippage as an underlying cause\textsuperscript{123,124} suggesting that corneal thinning is possible by tissue redistribution as opposed to tissue loss.\textsuperscript{125} This contrasts with theories of reduced tissue maintenance (keratocyte apoptosis, enzymatic degradation and oxidative stress) however, it is possible that reduced tissue maintenance may contribute to reduced inter-lamellar forces and hence, lamellar slippage which may contribute to reduced mechanical strength in keratoconus.\textsuperscript{126}

2.3.3.3 Age of Onset

Nottingham postulated two other theories on the origin of conical cornea; the first being that it is a congenital defect. He theorised that the cornea is most prominent at 12 weeks gestation, more so than any other period of pre-natal or post-natal life, and that some disturbance halts the development of the cornea at this stage, leaving it conical in shape throughout life.\textsuperscript{76} His second theory being that the defect is acquired in puberty, he postulated that it is known that at this stage of life, refractive abnormalities of the eye, such as myopia, occur. Nottingham attributed the acquired
myopia to an increase in corneal curvature and when this process continues, progressing beyond usual myopia, conical cornea results.26 We now know that the onset of myopia during the years of puberty is usually due to an increase in axial length rather than an increase in corneal curvature. In addition to these two theories Nottingham made a rather bizarre connection, indicating that individuals with a conically shaped head or “sugar-loaf or conical cranium” as he described it are more likely to be affected by keratoconus, though he did debate whether this connection truly existed.26 It is possible that Nottingham had come across individuals with craniosynostosis such as oxycephaly, who had survived a premature birth and mistakenly labelled myopia, the most common refractive error in premature births,127 with conical cornea.

Overall Nottingham’s ideas and observations on the origin of conical cornea should be cautiously compared. It may be that Nottingham in his comments on altered corneal “nutrition” was alluding to possible microstructural, cellular and biochemical anomalies that have subsequently been implicated in the underlying mechanism of keratoconus. However, his other theories such as the involvement of “active” inflammation and keratoconus being a congenital defect are largely incorrect.

2.3.3.3.4 Genetics and the Environment
Nottingham made several clear inferences of keratoconus being a hereditary condition, in one instance stating that the condition affected “several children born of the same parents, and not unfrequently, several members of the same stock in successive generations” and a case series by von Ammon concerning several sisters with the condition is relayed.26 Additionally he provided a number of arguments implicating environmental insults that may cause the disease. Nottingham believed the larger number of keratoconus cases in China to be due to the climate, meteorological conditions and habits.26 He specifically isolated the apparently cleanly habits of the Chinese, in one instance reporting that “the injurious effects of an every-day practice of the Chinese barbers, who cleanse or "wash" the eyes of the people with an ivory or bamboo instrument, shaped like a small scoop, which they pass under the lids and deeply into the canthi; this " operation" leaves the eye red and irritated, may have been related to conical cornea.26 Nottingham also queried whether the smoke in the huts of the Chinese and Northern Europeans precluded them to keratoconus.26

Since the time of Nottingham, the hereditary component of keratoconus has been well documented with diverse international studies reporting a family history of disease in between 5 - 20% of subjects.3,48,128 Further compelling evidence of the genetic component of keratoconus is conveyed by the fact that it has been observed in monozygotic twins.129,130 The relatively low familial inheritance might suggest that keratoconus is an autosomal recessive disorder or autosomal dominant with variable penetration; however, corneal topography of family members of patients...
with keratoconus reveals characteristics of astigmatism or mild/forme fruste keratoconus in up to 57% of cases,\textsuperscript{131, 132} suggesting an autosomal dominant predisposition with environmental factors playing a role in pathogenesis. It has also been demonstrated that clinically manifest keratoconus can occur in one but not the other twin, in a pair of monozygotic twins,\textsuperscript{129, 130} indicating that exogenous factors may indeed have an influence.

A number of environmental factors are associated with the development of keratoconus, including atopic eye disease, such as vernal keratoconjunctivitis (VKC) and allergic conjunctivitis, which are associated with more severe keratoconus.\textsuperscript{133} Though atopy is related to a person’s susceptibility, the occurrence and severity is determined by the environment, VKC for example occurs in warm climates, where it tends to be more severe.\textsuperscript{134} Other exogenous associations of keratoconus include contact lens wear and eye rubbing,\textsuperscript{3, 48} both of which are implicated in the pathological mechanisms discussed already. Interestingly, eye rubbing may not be so different from the eye washing procedure described by Nottingham.

A number of genes and loci have been proposed to play a role in the development of keratoconus. Among these are the genes for; an isozyme of superoxide dismutase SOD1,\textsuperscript{135, 136} tissue inhibitor metallopeptinase 3 (TIMP-3),\textsuperscript{136} and genes for different types of collagen e.g. \textbf{COL4A3} and \textbf{COL4A4},\textsuperscript{137} however no link has been definitively proven. Keratoconus has also been linked to a number of genetic disorders, including; trisomy 21 (Down syndrome),\textsuperscript{138} and genetic connective tissue disorders such as Ehlers-Danlos Syndrome,\textsuperscript{139} and mitral-valve prolapse,\textsuperscript{140} providing further weight to the theory of an underlying genetic predisposition to keratoconus.

Nottingham clearly alluded to genetic and environmental factors as having a role in the pathogenesis of keratoconus but he did not make the connection that two were potentially linked.\textsuperscript{26}

\section*{2.3.4 Treatment}

Treatment modalities for keratoconus traditionally ranged from complex spectacle prescriptions, to rigid gas permeable contact lenses in the moderate stages, and ultimately corneal transplantation in the end stages. Recently, new treatments aimed at halting the progression of keratoconus, such as corneal collagen cross-linking (CXL), have emerged. In his preface, Nottingham suggested a similar treatment algorithm; \textit{“The treatment: in some measure for the purpose of showing that surgical operations can only be of service in a few exceptional cases, and that optical instruments, in the earlier stages, or less developed forms of the disease, may be of great use.”}\textsuperscript{26} Nottingham characterised four relatively broad categories for the treatment of keratoconus, practised by him and others; Optical, Medical, Surgical and Mechanical. He notes the variability in success of these
treatment modalities; “It is worthy of remark, that scarcely any two practitioners have been equally fortunate with the same remedial means” indicating the difficulty with which keratoconus was treated.\textsuperscript{26} Here we discuss the treatments illustrated by Nottingham and their limitations, comparing them to those currently employed.

2.3.4.1 Optical

Nottingham described a number of optical management options for conical cornea. In many of the cases presented, he described many patients as extremely short sighted, not being able to discern objects in the distance but are able to visualise objects at near. He correctly surmised that the increased corneal curvature would increase its refractive power. As a result, Nottingham prescribed “bi-concave” lenses, in many instances with reported success.\textsuperscript{26} Astigmatism was first described by Thomas Young in his paper “On the mechanism of the eye”, presented to the Royal Society in 1800.\textsuperscript{60} Similarly, Nottingham inferred that a conical cornea had two curves, the greater of which was in the vertical meridian, what we now call with-the-rule astigmatism, and that a cylindric lens with its curve oriented vertically could account for the greater corneal refractive power in the vertical meridian.\textsuperscript{26} Later he alluded to the use of two cylindrical lenses “with their curves crossing one another” or axes perpendicular being beneficial in some cases.\textsuperscript{26} He also alludes to the use of what may be a form of Galilean telescope, used for improving vision; “Combinations of lenses of different figures, the ocular one bi-concave, the distal convex, with adjusting apparatus”, additionally making mention of an apparatus used by Dr Hull, fashioned by Mr Abraham, an optician, “formed of two lenses, with an adjustment. The farthest and largest lens is convex. The lens near the eye is smaller and doubly concave”.\textsuperscript{26} This is a method of optically magnifying objects, enlarging them so that an eye with reduced resolving power, as in a case of keratoconus, can visualise them, as opposed to a method of managing the optical disturbance caused by keratoconus.

Finally, Nottingham also describes the optical efficacy of pin-hole goggles, each half of which is made of “a piece of black wood, in form more or less like the one-half of a walnut shell”,\textsuperscript{26} or in other words a sphere cut in half. These hemispherical eye coverings were perforated either centrally or eccentrically. Nottingham suggested them to be placed nasally so as to be in the correct position when the eyes converged when viewing a near object, and sometimes had a tubular elongation, protruding anteriorly.\textsuperscript{26} These goggles would improve the vision of a person with keratoconus, as the pin holes would somewhat overcome the optical issues induced by the shape of the keratoconic cornea depending on the magnitude of ametropia, however, this technique would severely restrict a subject’s visual field.
It is of course, not beyond the realms of possibility that Nottingham was including a few cases of extreme myopia, or myopic astigmatism. In supporting this conjecture, in some cases presented, simple bi-concave lenses appeared to completely relieve the patient’s symptoms. Without a record of slit-lamp signs, corneal curvature, and visual measures such as visual acuity, it is difficult to judge if Nottingham was, at least in some cases, discussing myopia and not keratoconus.

2.3.4.1.1 Contact Lenses
Of all the optical management options discussed by Nottingham, none truly address the major underlying cause of severe vision loss in keratoconus, that of an irregular corneal shape. The early stages of keratoconus where corneal astigmatism is still fairly regular continue to be managed with spectacle correction in modern times. However, as the disease progresses and corneal astigmatism advances, increasing in magnitude and becoming progressively more irregular, spectacles no longer provide adequate vision. Contact lenses are then the only optical treatment modality that can effectively address the irregular corneal shape, characteristic of keratoconus.

Surprisingly, glass corneo-scleral shells were developed independently and simultaneously by Eugen Fick (Zurich, Switzerland), Eugene Kalt (Paris, France), and August Müller (Keil, Germany) and presented in 1888 or early 1889. Both Fick and Kalt developed their contact shells for the treatment of keratoconus, at least in part, whereas Müller developed his for the correction of his own myopia. Since these devices contained no specified refractive power, technically they should be considered contact shells as opposed to contact lenses (CLs).

Considering that these contact shells were produced over 30 years after the publication of his treatise, Nottingham made limited comment in regard to contact lenses or shells as a treatment option for keratoconus. He does however allude to the potential use of such devices in a single statement; “Lenses with posterior surface corresponding to the front of the eye, and anteriorly of regular figure, are amongst the means proposed for correcting the altered refraction; along with these may be mentioned lenses of transparent animal jelly, contained in capsules of glass, to be placed on the front of the eye”.

In contemporary practice, soft CLs made of hydrogel or silicon-hydrogel are the most comfortable option and can provide a more regular refracting surface and correct relatively large amounts of astigmatism. Indeed, in recent years a number of specialty soft CLs have been designed specifically to treat keratoconus. These lenses generally have a steeper base curve with flattening of the para-central curves to match the eccentricity of the keratoconic cornea. This type of lens has been shown to have comparable results to that of rigid gas permeable (RGP) contact lens in treating non-
surgical corneal ectasias. The vision of patients with moderate keratoconus cannot be corrected adequately with soft CLs, however, RGP CLs maintain their shape on the eye and combine with the tear film to produce a uniform refracting surface and an RGP lens can be designed to fit most corneas; however, the major short and long–term issue with RGP contact lenses is that of comfort. To overcome this issue piggy-back systems where the patient wears two lenses, a RGP lens on top of a soft lens, have been developed. More recently, hybrid CLs, consisting of a rigid centre and a “soft skirt”, have been developed to simplify the issue, and have been shown to produce similar visual outcomes to rigid lenses but with superior comfort.

Since the development of contact shells in the late 19th century, CLs have seen an explosion in their development that began in the late 20th century and continues today. CLs have become a staple in the management of keratoconus and are likely to continue to do so, especially with the advent of intra-corneal ring segments and CXL, both of which may play a role in maintaining patients in contact lenses and preventing or delaying corneal transplantation.

2.3.4.2 Medical
Nottingham writes of a variety of systemic and topically applied medications. Oral medications include “sulphate of zinc” in combination with “sulphate of magnesia”, arsenic, “tincture of sesqui-chloride of iron with aloes and myrrh” among others. Topical applications of the “nitrate of silver, in substance” is proposed to prevent corneal opacification, “acetate and sulphate of zinc” and lead in a lotion, “nitric-oxide of mercury” in ointment, “vapour of prussic acid” to treat corneal opacification, apparently reversing it, and Belladonna or atropine, to mention a few.

In modern medicine, no such remedies are utilised as they have never been proven to treat keratoconus. Indeed, there have been no useful advances in medical treatments for keratoconus per se. However, recent research as previously noted, by identifying molecular components that are implicated in keratoconus, may provide potential targets for futures therapeutic interventions.

2.3.4.3 Surgical
Nottingham’s treatise discusses a great many surgical procedures for the treatment of keratoconus, performed by him and others. However, he was reluctant to advocate any surgical procedures, considering their limited success rate, and he advocated their use only when all other options had been exhausted; stating: “It is obvious that, before proceeding to surgical operation, the different forms of optical contrivance should be resorted to, and their various combinations carefully tried when the more simple ones fail to assist vision”. Surgical interventions in the mid-nineteenth century were limited by surgical instrumentation and technology, a lack of therapeutic agents such as
antibiotics and steroids, and limited knowledge of the anatomy and physiology of the eye. However, some of the surgical procedures discussed illustrate a reasonably accurate understanding of keratoconus.

Early in his treatise, Nottingham discussed a surgical technique that is similar to anterior chamber paracentesis. In the procedure a needle is used to puncture the cornea and aqueous humour is allowed to escape thus flattening the cornea due to a reduced intra-ocular pressure. This technique was performed by Walther, and an alternate technique was proposed by Fario, which involved removal of part of the cornea coupled with applying pressure, to allow continued extrusion of aqueous humour, both authors reported varied treatment success. One of the inherent flaws with this technique is that the cornea quickly seals this “drainage channel”, meaning this is a temporary treatment and would need to be repeated, something which Nottingham notes. In addition to its temporary nature, scarring of puncture sites (if centrally placed) could obscure vision; however, eccentric puncturing was suggested where possible.

As mentioned in preceding sections, Nottingham proposed that keratoconus only affected the central cornea, leaving a normal peripheral cornea. Thus, he proposed to create an eccentric artificial pupil in order to improve visual function. Unsurprisingly this procedure would have limited success, as light through a peripheral pupil would fall on correspondingly peripheral retina with poorer resolving power than the macula. Other surgical interventions suggested include; corotomia or excision of the conical part of the cornea, division of the recti muscles to alter abnormal action on the cornea and removal of part of the cornea to promote scarring and corneal flattening, all of which met with little success and do not have comparable procedures in modern times.

2.3.4.3.1 Refractive Surgery
An interesting technique proposed to treat keratoconus by Nottingham was dislodging of the crystalline lens by couching, either when the lens was clear or cataractous. This procedure met with questionable success but Nottingham correctly postulated that removal of the lens and hence its refractive power, would counteract the increased refractive power of the cornea in keratoconus. In recent times, surgical refractive options for correcting the myopia and astigmatism that occurs with keratoconus have been explored with relative success. Ablative corneal refractive procedures using an excimer laser such as photorefractive keratectomy (PRK) or laser-assisted in situ keratomileusis (LASIK) have up until relatively recently, been absolute contraindications in forme fruste or keratoconus cases (due the risk of subsequent keratectasia). However, recently, PRK has been explored as a laser surgical refractive treatment option for keratoconus as it leaves a greater residual
stromal thickness than LASIK. PRK has been shown to improve unaided vision and topographic features in forme fruste\textsuperscript{148} and mild-to-moderate keratoconus,\textsuperscript{149} with no keratectasia.

Intra-ocular lenses (IOLs) are another surgical refractive option in keratoconus and these somewhat mimic the refractive change of removing the crystalline lens suggested by Nottingham. Anterior and posterior chamber phakic IOLs, which are available in toric form, are appropriate for young patients with keratoconus who still have use of their accommodation\textsuperscript{150,151} and can be effective in correcting the myopia and astigmatism in mild-to-moderate keratoconus. As patients with keratoconus age and become presbyopic or develop cataracts, removal of the crystalline lens and replacement with an artificial lens is recommended (pseudophakia). Traditionally, pseudophakic IOLs were capable of only correcting the spherical component of a patient’s refractive error; however, toric forms of these lenses have become increasingly available. IOLs are effective in correcting vision in mild-moderate keratoconus.\textsuperscript{152,153} Phakic and pseudophakic IOLs have the same inherent limitation as spectacles in treating keratoconus; they do not address the underlying issue of corneal irregularity and typically can only be used when keratoconus is mild to moderate with largely orthogonal astigmatism. While Nottingham may not have been able to predict the advances in surgical refractive technology, he correctly deduced that the refractive power of the eye could be altered surgically to improve vision in keratoconus.

2.3.4.3.2 Corneal Transplantation

Ultimately, in progressive keratoconus when contact lenses no longer provide adequate vision correction corneal transplantation is the mainstay of treatment. There are two general types of corneal transplantation currently used for keratoconus; penetrating keratoplasty (PKP) where the entire thickness of the cornea is replaced, and deep anterior lamellar keratoplasty (DALK) where the host Descemet’s and corneal endothelium are retained.

Nottingham makes mention of corneal transplantation as a surgical treatment for keratoconus only twice in his treatise. The first of these two comments being; “There is a work by Tome, of Bonn, on the transplantation of the cornea, which may be consulted by those who are likely to take interest in such a branch of surgery.”\textsuperscript{26} The reference states “Neither transplantation nor glazing of the cornea seems to have been seriously thought of in this country, as ever likely to repay the trouble and suffering associated with their performance; if such operations were rarely capable of supplying a transparent front to the eye, devoid of annoyance, and of which the patient could avail himself in vision, their repetition would, doubtless, be desirable, and cases of conical cornea, accompanied by opacity, might occasionally lend themselves to the attempt; but with regard to results of practice in this department of art, the expectations of surgeons are not yet likely to be very sanguine.”\textsuperscript{26} Thus
Nottingham put forth that the idea of corneal transplantation may have the potential to be a credible treatment option, however, his doubt about current success may have been influenced by Himly, who at the time advocated xenograft corneal transplantation, from a “a dog, pig, or other animal”, which understandably did not receive much success. However, around the time of the publication of Nottingham’s treatise, allografts such as that performed by Samuel Bigger on a pet gazelle and lamellar approaches by the likes of Arthur Von Hippel were somewhat successful.\textsuperscript{154}

It was not until 1905, just over 50 years after the publication of Nottingham’s treatise, that the first successful corneal transplantation in humans was performed by Eduard Zirm.\textsuperscript{154} Since its development, PKP has been used successfully to treat end stage keratoconus (Figure 2-9 A and B). Graft survival, complication rates, visual and refractive outcomes have consistently been reported as exceptional.\textsuperscript{155-157} In recent times, DALK has gained popularity as alternative to PKP in treating keratoconus, as it provides the allure of being essentially an extra-ocular procedure, keeping the host endothelium intact. Thus there is a theoretically reduced risk of graft rejection and intraocular complications such as endophthalmitis and expulsive haemorrhages.\textsuperscript{158} Very long-term follow up of DALK for keratoconus is not currently available; however, existing data suggests that compared to PKP, graft survival, visual and refractive outcomes are similar while there are fewer post-operative complications\textsuperscript{159, 160}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{penetrating_keratoplasty.png}
\caption{Penetrating keratoplasty where the indication was keratoconus through a slit-lamp biomicroscope \textbf{A}. A healthy penetrating keratoplasty with interrupted sutures in place – 6 months post-operative \textbf{B}. A healthy penetrating keratoplasty – 4 years post-operative}
\end{figure}
2.3.4.4 Mechanical

Mechanical alteration of corneal shape is an intriguing and intuitive method of treating conical cornea proposed by John Nottingham. In relation to a case of conical cornea with no opacification he claims; “the prominence of the cornea could be flattened in but a very small degree, by any mechanical agency that would not injure the surface of the tunic, such flattening might at once lead to improvement of the sight, which would be likely to continue as long as the modification in the form of the cornea could be sustained.” 

Nottingham relates a case in which he specifically utilised a mechanical contrivance in the correction of myopia. He notes the closure of the palpebral aperture often observed in short sighted individuals when attempting to view near objects. Nottingham suggested that this action not only acts as a pin-hole but “backward pressure” from the eye-lids flatten the cornea, albeit not quite enough to overcome the short sightedness completely. He suggested artificial addition to the action of the lids using a mechanical device to further flatten the cornea. This device consisted of “a steel spring, with a small round pad” that is “lined with a soft material, being placed upon the temporal integument”, near the temporal canthus which has been previously drawn backward. This spring passed “downwards and forwards, towards the canthus upon which it has to act” and was held in place by another spring “encircling the fore-part of the head, in shape like a horse shoe, and completed by an elastic band carried round the occiput, so as to form a kind of fixed and steady head-band”. This device was reported to alleviate the myopia of a number of patients including a sailor and clergymen. The effect of the device is however not just mechanical as the juxtaposition of the eye-lids also act as a pinhole. Such a device could not be a viable treatment option as it would likely cause great discomfort in a short period of time and would no doubt carry a heavy social stigma that would preclude its use in most cases.

Though Nottingham’s mechanical contrivance for altering the corneal shape was not a viable treatment option, technological development has produced more applicable methods of mechanically modifying the corneal shape. Intra-corneal ring segments (ICRS) such as Intacs (Addition Technologies, Sunnyvale, CA, USA) are semi-circular polymethyl methacrylate(PMMA) corneal implants. ICRS, like Nottingham’s device, were initially proposed as a method for myopia correction, by Reynolds in 1978, and subsequently applied to ectatic conditions such as keratoconus. A surgical channel is made in the stroma at 70 – 80% corneal thickness either mechanically using a diamond knife or with a femtosecond laser, the two semi-circular rings are then inserted, one superior and the other inferior. ICRS act to mechanically flatten the cornea centrally. A number of studies have reported improvements in refractive and visual outcomes and topography following ICRS implantation in eyes with keratoconus, however, they do not prevent disease.
ICRS may play a role in delaying or preventing corneal transplantation in keratoconus, the alteration in corneal shape has been shown to not only improve spectacle corrected vision, but also contact lens comfort in intolerant patients.167-169

2.3.4.4.5 Corneal Collagen Cross-linking

CXL is a treatment aimed at preventing further deterioration of the corneal shape in keratoconus and a study of the safety and efficacy of two accelerated CXL protocols is detailed in chapter 9. Indeed, CXL has been shown to flatten the cornea slightly post-operatively. While it does not significantly alter the shape of the cornea mechanically, it does alter the mechanical properties of the cornea, stiffening it to prevent further disease progression. As previously mentioned, much of the effect of keratoconus is due to biomechanical changes in the cornea which have been linked to corneal thinning and a reduction in the number of cross-links within and between collagen fibres in the corneal stroma.170 CXL was first introduced in 1998 by a group at Dresden University as a possible procedure to improve the biomechanical strength of the cornea by increasing the number of cross-links in the corneal stroma171 and was first clinically explored to prevent the progression of keratoconus in 2003.13 The procedure of CXL consists of applying riboflavin to the cornea and irradiating it with ultraviolet-A (UVA) radiation. The UVA excites riboflavin into a triplet state, inducing the production of reactive oxygen species, which in turn induces covalent cross-linking of corneal stromal molecules including collagen and proteoglycans.172, 173

Analysis of the stress-strain relationship of ex vivo human corneas treated with cross-linking reveals an increase in rigidity, suggested by a 328.9% increase in stress and increase in the stress-strain relationship (Young’s modulus) by a factor of 4.5.174 Peculiarly, in vivo analyses show no significant difference pre and post treatment.175-177 This may be due to the fact that devices such as the ORA and CorVis ST do not measure true biomechanical properties but instead uses surrogate indices to indicate biomechanical properties. Wollensak et al.178 observed that cross-linking did not increase interlamellar cohesive forces, which may allow for interlamellar sliding under the air-puff of the ORA.

A number of clinical trials have been carried out with a focus on the effects of CXL and its role in preventing the progression of keratoconus. These trials suggest that cross-linking halts the progression of keratoconus in most cases and it appears that in successful cases, progression is halted for at least 3 years,179-182 though observational investigations suggest CXL may be effective at halting disease progression for 7-10 years.183, 184 Therefore, CXL may prove a useful tool in delaying or even preventing the need for corneal transplantation in certain cases, indeed CXL has been shown to reduce the transplantation rate for keratoconus when implemented long term.185 As previously mentioned, Nottingham did allude to the reduced corneal biomechanical properties in keratoconus;
however, this was more of an observation than a causal link. Therefore it is unlikely that he may have foreseen that a procedure to increase the rigidity of the cornea would be a possible treatment option for keratoconus.

Nottingham described how the treatment of keratoconus often requires a combination of interventions in order to be managed with success; “Remedies administered by way of the stomach, topical applications to the eye and neighbouring parts, optical contrivances to assist the vision, surgical operations performed on the eye-balls, with the various combinations of these, may be said to include the main proceedings hitherto suggested; in connection with which it must be remarked, that in some cases benefit is only to be derived from a judicious combination of general and local treatment, or of operation and after-treatment”.26 This still rings true today where multiple interventions are often combined to achieve the best outcome for patients. Numerous examples can be provided with combinations of CXL and PRK, IOL and RGP contact lens, ICRS and RGP contact lens being just a few.

2.3.5 Conclusions

We live in an exciting era where technology advances at a blistering pace, allowing medical science to examine clinical manifestations and underlying mechanisms of disease in a way that could not have been dreamt of by the observers who, over a century ago, described many of the diseases that are now under our attention. This resounding truth is not lost when we consider keratoconus. Many of the observations by pioneers such as John Nottingham still ring true today. Considering the vivid detail and relative accuracy of the description of keratoconus by John Nottingham, he may well be the first person to accurately describe the condition despite his shortcomings, which have only been overcome by progressively increasing knowledge and improving technology. In this age of technologic wonder and research, we must not forget the foundations laid by our predecessors that have allowed us to reach such an intricate understanding of the disease known as keratoconus.
2.4 Keratoconus in New Zealand

The previous section of this chapter (2.3) compared the knowledge amalgamated by John Nottingham to that of the late 20th and early 21st century, along the way, highlighting some of the triumphs in our collective understanding of keratoconus but also our shortcomings, illustrating areas that require further attention, namely; epidemiology, natural history and prognosis, pathogenesis and treatment.

NZ is a unique location to investigate keratoconus as it is believed that the condition is more prevalent, particularly among individuals of Māori and Pacific Peoples descent. There has been a significant amount of research into epidemiological and clinical characteristics of keratoconus in NZ, much of which has been summarised in a recent review by Patel and McGhee.186 The purpose of this chapter is to explore the current understanding of the epidemiological and clinical characteristics in NZ and the shortcomings in the understanding of these aspects of the condition.

2.4.1 Epidemiology

The only direct investigation of the epidemiology of keratoconus in NZ was conducted by Sabiston in 1978, where he stated that ‘The occurrence rate in Hawkes Bay has been calculated to be one in two thousand of the population and is found to be equally present in both Maoris and Europeans’.12 Interestingly, there are no further investigations directly studying the prevalence of keratoconus in NZ, however, several studies have included analyses of the demographic characteristics. It is noteworthy that there has been a longstanding clinical impression that keratoconus is of greater prevalence in NZ and has an ethnic predilection for individuals of Māori and Pacific Peoples descent.

A large practitioner-based survey revealed that most subjects with keratoconus in NZ were of European and Māori or Pacific Peoples descent, with those of Māori or Pacific Peoples accounting for approximately 20% of the cohort. Interestingly, the ethnic distribution of the cohort with keratoconus was similar to that of ethnicity proportions in the general NZ population.187

In contrast, other investigations suggest that Māori and Pacific Peoples ethnicities are disproportionately over-represented in the keratoconus population. Indeed, a recent study by Jordan et al.39 revealed that patients of Māori and Pacific Peoples ethnicities were over-represented in the cohort of patients with keratoconus attending subspecialty cornea and external disease clinics at the Department of Ophthalmology, Auckland District Health Board, and European and Asian patients were under-represented, compared to the total population of patients attending the ophthalmology service overall. Screening of teenagers attending two specifically selected high schools in the Waikato region of NZ for topographic corneal anomalies suggested that topographic patterns
suggestive of early keratoconus affected 19% the screened cohort. While these results are not directly comparable to similar international investigations in terms of the age and type of populations screened, methods of screening employed and diagnostic criteria utilised; they are suggestive of a higher prevalence of keratoconus in NZ (19% vs. 2.34% (Israel), 2.30% (India), 0.09% (Denmark), and 0.05% (United States of America (USA))). Furthermore, screening of these high school students in NZ for topographic indications of keratoconus revealed that the proportion of Māori/Pacific Peoples students exhibiting suspect corneal topography was twice that of non-Māori/Pacific Peoples students.

Further evidence of a potentially higher prevalence of keratoconus in NZ compared to other parts of the world is suggested by the previously reported data on the proportions of corneal transplantations performed for keratoconus. In NZ, 45.6% of corneal transplantations from 1991-1999 and 41.1% from 2000-2009 were performed for keratoconus, compared to 31.0% (Australia), 18.6% (Hungary), 13.8% (Canada), 22.5% (United Kingdom), and 16% (USA). Other possible factors contributing to the particularly high rate of corneal transplantations performed for keratoconus in NZ include the fact that Māori and Pacific Peoples comprise approximately 20% of the total population and the aforementioned studies suggest that keratoconus is more prevalent in these ethnic groups. Furthermore it is possible that keratoconus is particularly progressive in NZ, with subjects progressing to the stage where corneal transplantation is required more rapidly. Interestingly, keratoconus is also the leading indication for corneal transplantations in NZ performed on patients aged ≤14 years (67.2%), considerably higher than reported internationally (0–11%).

2.4.2 Clinical Characteristics of Keratoconus

A large scale clinical investigation of the keratoconic population in NZ demonstrated that 13% exhibit corneal stromal scarring, 38% a Fleisher ring and 17% Vogt’s striae. Based on axial keratometric topographic maps, the cone shape has been predominantly described as asymmetric bowtie (296 (42%)), followed by round (186 (31%)). Furthermore, Owens and Watters observed that each subject typically exhibited the same cone shape in both eyes, however, the investigation by Jordan et al. previously mentioned suggests that complete enantiomorphism based on topography may only be observed in as little as 12.5% of subjects with keratoconus and the prevalence of forme fruste keratoconus may be as high as 12%. The use of ultrasound pachymetry in early investigations revealed that corneal thickness was maximally reduced in an area approximately 2.4 mm below the visual axis and that even in severe keratoconus, the superior cornea does not experience significant thinning. Additionally, an inferior-superior (I-S) difference in pachymetry of ≥100 µm was considered as pathognomonic of keratoconus, with three categories of I-S pachymetric differences
indicative of disease severity: suspect/mild keratoconus at 80-100 µm, moderate keratoconus at 100-125 µm and advanced keratoconus at ≥125 µm.\textsuperscript{191}

Several investigations utilising \textit{in vivo} confocal microscopy (IVCM) to characterise the corneal microstructural alterations that occur in keratoconus have been conducted in NZ. Indeed, the previously mentioned investigation of the distribution of sub-basal nerves in the living keratoconic cornea utilising a wide-field montage technique conducted by Patel and McGhee in NZ was the first study of its kind.\textsuperscript{118} The results of this study confirmed that the pattern of distribution and density of the corneal sub-basal nerve plexus is altered even in the early stages of keratoconus. Sub-basal nerve fibre bundles demonstrated an abnormal configuration at the corneal apex, where these bundles appeared to consist of a tortuous network, many of which formed closed loops. Additionally, these nerve fibre bundles appeared to conform to the topographic shape of the base of the cone, with many bundles appearing to run concentrically with the cone in this region.\textsuperscript{118}

The altered distribution and density of the corneal sub-basal nerves in keratoconic eyes provides substantial evidence that the corneal sub-basal nerves are involved in the keratoconus disease process. Furthermore, studies investigating corneal sensation using a quantitative non-contact pneumatic corneal aesthesiometer\textsuperscript{94} and IVCM\textsuperscript{94, 192} in NZ provide further evidence for the involvement of the sub-basal corneal nerves in the keratoconus disease process. The results of these studies suggest that the sub-basal nerve density and basal epithelial density are significantly lower in keratoconic corneas compared to normal corneas; however, while the central corneal sensation was only reduced significantly in contact lens (CL) wearing keratoconics compared to normal subjects, there was no difference between the non-CL wearing keratoconic and normal groups. IVCM investigations in NZ have also confirmed that keratocyte density is significantly reduced in subjects with keratoconus when compared to age-matched controls.\textsuperscript{192, 193}
2.4.3 Conclusions

The previous investigations of keratoconus in NZ suggest that keratoconus is both more common than in many countries internationally and also more common within the Māori/Pacific Peoples populations. However, further investigation is required as much of the data concerning the prevalence of keratoconus in NZ is inferred from investigations not specifically investigating the epidemiology, thus highlighting the need for specific epidemiological investigations.

Investigations of the clinical characteristics of keratoconus in NZ have made several novel contributions to the knowledge of keratoconus overall. However, it is particularly noteworthy that considering it is possible that keratoconus in NZ is of a more progressive form, no natural history investigations of keratoconus have been conducted in NZ, such as the CLEK² and DUKS³ studies. Thus assessment of how the corneal phenotypic characteristics of keratoconus change as the disease progresses, and the rate at which these changes occur, has not been investigated directly. Therefore it is pertinent to conduct a large scale, prospective, longitudinal investigation of the natural history of keratoconus in NZ.

With regards to treatment, it is surprising that only a single investigation related to corneal collagen cross-linking (CXL) has been conducted in NZ.¹⁹⁴ Considering that keratoconus is believed to be more common in NZ, it is an ideal location to conduct large scale prospective investigations of the safety and efficacy of CXL and variations in the protocol of this procedure.
Chapter 3:

Methodology
This chapter details the devices and techniques utilised to conduct the investigations concerning keratoconus detailed in chapters 4-10. The technical aspects of the devices and methodology used to obtain the desired outcome measures are appropriately described. These devices and techniques include: unaided vision and visual acuity, refraction, assessment of corneal power/shape and thickness, corneal biomechanics and corneal microstructure. The main characteristics of keratoconus that have previously been elucidated using these devices are detailed in chapter 2. All instruments detailed are located within the clinical research laboratory located within the Ophthalmology Department, Greenlane Clinical Centre, Auckland, New Zealand, except the Medmont E-300 which is located at Mortimer Hirst Eyecare and Eyewear, Auckland.

Techniques for Assessing Disease Characteristics

3.1 Unaided Vision and Visual Acuity

Measurement of the level of detail an eye is able to resolve is essential to quantify the effect of a disease on a participant’s vision. Measurement of the resolving ability of the eye is generally carried out utilising specially designed letter charts.

Unaided vision generally pertains to resolving ability of the eye without refractive correction while visual acuity indicates that the resolving ability of the eye was measured with refractive correction in place (either spectacles or contact lenses).

In chapters 5 and 9 unaided vision and visual acuity were measured using a Bailey-Lovie chart (Figure 3-1) at a 3m testing distance under standardised lighting conditions. In chapters 4, 6 and 7 unaided vision and visual acuity were measured utilising various different vision charts; projected charts, digital charts and traditional charts such as the Bailey-Lovie, at 3m or 6m test distances.

Unaided vision and visual acuity were recorded using the standard Snellen notation using letter by letter scoring and subsequently converted to LogMAR for statistical analysis.
3.2 Spectacle Refraction

Spectacle refraction is a means of measuring the refractive error or defocus of the image produced by the refractive media of the eye, relative to the retina. For the studies in this thesis, retinoscopy or auto-refraction was used as a starting point followed by the standard subjective refraction procedure in order to determine refractive error.

3.3 Slit-lamp Biomicroscopy

The slit-lamp biomicroscope is an invaluable tool that emerged in the early 20\textsuperscript{th} century (chapter 2) and forms the cornerstone of the modern clinical ophthalmic assessment. The slit-lamp also revealed many of the characteristics of keratoconus (chapter 2) as it enables the operator to obtain a high magnification stereoscopic view of the ocular tissues that make up the anterior segment.

3.3.1 Technical aspects of the Slit-lamp Biomicroscope

The slit-lamp consists of a microscope, coupled with an illumination system (Figure 3-2A.). The microscope and illumination system can rotate independently but both components rotate around the same axis, thus the system is parfocal; the slit beam and microscope remain in focus regardless of their relative positions.\textsuperscript{195} The illuminating mechanism is composed of a light source (6V/20W halogen lamp), a system of condensing lenses, and an adjustable diaphragm that permits variations in slit width and height, ranging from 0-14mm.\textsuperscript{196} Several illumination settings are available including: neutral density filters to adjust the intensity of the beam, a red-free filter which eliminates the red wavelengths thus produces a green beam and a cobalt blue filter which produces a blue beam of light. The microscope system is composed of two convergent binocular tubes containing Galilean
3.3.2 Utilisation of the Slit-lamp Biomicroscope

The participant is asked to place their chin on the chin rest and forehead against the forehead bar (Figure 3-2B.). The height of the chin rest is altered to align the participant with the outer canthus marking (Figure 3-2B.) and the table height is altered in order to make the participant comfortable. Once a participant is in place, several examination techniques are used to assess the structures of the anterior segment of the eye. Various characteristics of the keratoconic cornea are particularly apparent when utilising some of these techniques (detailed below).

**Figure 3-2** The Haag-Streit slit-lamp biomicroscope (Haag-Streit, Koeniz, Switzerland) A. Haag-Streit slit-lamp biomicroscope with attached Canon digital camera B. Haag-Streit slit-lamp biomicroscope in clinical use

**Diffuse Illumination**

Diffuse illumination utilises a large unfiltered or neutral density filtered beam and can illuminate the entire cornea simultaneously. Diffuse illumination enables detection of gross abnormalities such as the anterior corneal stromal scarring associated with advanced keratoconus but is poor for defining fine details such as Vogt's striae or determining the depth of corneal lesions.\(^{197}\) Use of the cobalt blue filter with diffuse illumination is often used to better visualise Fleisher’s ring in keratoconus.
Direct Focal Illumination

Direct focal illumination, also known as parallelepiped, is achieved by using a narrow vertical slit beam, angled by moving the illumination to one side with respect to the microscope. When a very narrow beam is utilised, this technique produces an optical section which reveals the thickness of transparent or translucent structures. This technique is useful for detecting corneal thinning apparent in advanced keratoconus, indicates the depth of features such as stromal scarring, and demonstrates contours including the increased curvature of the cornea in keratoconus.195, 197

Retroillumination

Retroillumination requires that the slit-lamp is focussed on the structure of interest, while the slit beam is moved laterally to illuminate the region directly behind the structure of interest (indirect illumination). The beam reflected by the posterior structures produces a silhouette of the structure of interest, allowing subtle details not visible under direct illumination to be observed.195, 197

Sclerotic Scatter

Sclerotic scatter is a technique that utilises indirect illumination and is produced by directing the slit beam perpendicular to the surface of the eye, just adjacent to the corneal limbus. This set up produces total internal reflection of the light entering the corneal lamellae, akin to a fibre optic cable, illuminating the entire cornea from limbus to limbus. Sclerotic scatter enhances the visibility of subtle corneal opacities by scattering light from these opacities toward the observer.195, 197

Specular Reflection

Specular reflection requires that incoming light from a source is reflected by a surface in a single outgoing direction at the same angle with respect to the surface normal. In the case of the slit-lamp, the slit beam and biomicroscope are aligned so that their optical axes approach the cornea from opposite sides to the plane of the surface normal, producing equal angles of incidence and reflection. Use of 40x magnification and the specular reflection technique may allow individual corneal endothelial cells to be observed.195

Vital Dyes

Fluorescein is a vital dye commonly used in an ocular examination in conjunction with the slit-lamp biomicroscope and cobalt blue illumination. Fluorescein allows for visualisation of the tear film, and stains areas where the corneal epithelium is damaged or missing. Fluorescein is also useful in the assessment of an RGP contact lens fit as the amount of pooling beneath a lens is indicative of relationship between the corneal shape and posterior surface of the CL.
All studies described in chapters 4-9 of this thesis used various slit-lamp biomicroscopes, however, the technical aspects of different models are virtually identical, and the techniques described above were used to examine the corneas of participants included in the studies described in this thesis. The Haag-Streit (Haag-Streit, Koeniz, Switzerland) slit-lamp biomicroscope (Figure 3-2A. and B.) was utilised for the investigations carried out at Greenlane Clinical Centre, chapters 5, 8 and 9.

### 3.4 Computerised Corneal Tomography

Computerised corneal tomography has revolutionised our understanding of the characteristics of the keratoconic cornea, enabling detailed assessment of corneal power/shape and thickness profile.

#### 3.4.1 Orbscan II

##### 3.4.1.1 Technical Aspects of the Orbscan II

The Orbscan II (Bausch and Lomb, Rochester, NY, USA) computerised tomographer utilises a combination of Placido disc and slit-scanning technologies (Figure 3-3A). The Placido disc system consists of 40 monochromatic rings and analysis of the image of the rings, reflected specularly by the cornea (mires), in terms of size, shape and position relative to each other to provide data regarding anterior corneal power/shape. The slit-scanning system utilises 40 white light slits at an angle of 45°, distributed across the cornea; 20 from the left and 20 from the right, each measuring 12.5x0.3mm.198 The light from these slits passes through the cornea and is scattered in all directions, including back toward the device’s charge coupled device (CCD) camera, which images the slits.198, 199 The Orbscan II measures approximately 9,000 data points.198 The system utilises the principle of triangulation to analyse the slit images to directly assess both elevation data from the anterior and posterior corneal surfaces as well as corneal thickness. Further processing of the images allows for digital reconstruction of the anterior iris and lens. Of note, the triangulation process for the posterior corneal surface, anterior iris and lens is limited by the hardware, operator influence and influence of the accuracy of the anterior surface measurement, thus these measurements are not as accurate as that of the anterior corneal surface.199

##### 3.4.1.2 Utilisation of the Orbscan II

Acquisition of images begins in a similar fashion to the slit-lamp; the participant is asked to place their chin on the chin rest and forehead against the forehead bar (Figure 3-3D.). The height of the chin rest is altered to align the participant with the outer canthus marking (Figure 3-3D.) and the table height is altered to make the participant comfortable. Once in place, the participant is able to see the Placido disk and a central red fixation light (Figure 3-3A.). The participant is asked to maintain steady fixation on the red light within the instrument and to avoid blinking for the duration of the scan. The operator centres the mires of the Placido disk on the pupil centre to ensure x and y-
axis alignment and adjusts the distance to the eye until the alignment mires are correctly positioned (Figure 3-3B. and C.) to ensure z-axis alignment. The operator manually triggers the acquisition sequence once appropriate alignment is achieved. During acquisition, the Placido disk is illuminated and the mires reflected from the anterior corneal surface are imaged first (3 images total) (Figure 3-3B.). 198 Subsequently, 40 slits are projected onto the cornea, iris and lens and imaged. The acquisition process takes approximately 1.5 seconds. 198 The Orbscan II does not provide a quality score but automatically discards scans deemed to be of unacceptable quality.

Figure 3-3 The Orbscan II (Bausch and Lomb, Rochester, NY, USA) A. The Orbscan II with red fixation light and illuminated Placido rings visible B. Improper alignment of the Orbscan II, arrows indicate the directions to move the device to obtain proper alignment C. Proper alignment of the Orbscan II, arrow highlights the indicators of alignment, red circle centred on Placido ring mires (x and y-axis alignment) and z-axis alignment mires touching to form a “S” D. The Orbscan II in clinical use

The Orbscan II and the above method of acquisition, was utilised in the investigations detailed in chapters 8 and 9. The data obtained from the processed Placido disk and slit-scanning images are typically presented by the device’s software in a quad-map format (Figure 3-4). Axial or tangential power maps, anterior and posterior elevation maps, and pachymetry maps are the four most useful maps in assessing keratoconus. The axial power map provides information on power/radius of curvature of a given meridian across the cornea based on the optical axis of the measuring system.
Data on power/radius of curvature provided on the tangential power (instantaneous power) map are not limited to the optical axis of the device.\textsuperscript{200}

The anterior and posterior elevation maps provide data regarding the contour of the anterior and posterior corneal surfaces in relation to a best fit sphere. The in-built device software produces a best fit sphere (BFS) (radius of curvature in mm) for both the anterior and posterior surface; deviation in the contour of these measured surfaces from the BFS are represented as elevation above or depression below in micrometers.\textsuperscript{201}

The following parameters obtained from the Orbscan II were utilised as specified in chapters 8 and 9: steep simulated keratometry ($K_{\text{steep}}$) and flat simulated keratometry ($K_{\text{flat}}$) obtained from axial power, central corneal thickness (CCT) and thinnest corneal thickness (TCT).

**Figure 3-4** Standard quad-map of the Orbscan II (Bausch and Lomb, Rochester, NY, USA) in a participant with moderate keratoconus, displaying: axial power, pachymetry, anterior elevation and posterior elevation maps

Studies using Orbscan II suggest that central corneal thickness is overestimated compared to ultrasound pachymetry.\textsuperscript{202, 203} This overestimation is thought to be due to several factors relating to differences between the two examination techniques such as the lack of a tear film interface with the application of an ultrasound probe, tissue compression and alignment of the ultrasound probe. To compensate for this overestimation, an acoustic factor of 0.92 has been applied to pachymetry measurements obtained with the Orbscan II. However, this acoustic factor is a mean value which is
applied to all pachymetric measurements obtained with the Orbscan II and while this allows for correlation with measurements made using ultrasound, it is possibly less accurate in corneas that are particularly thick or thin.

Of relevance to this thesis, corneal thickness measurements in keratoconus with the Orbscan II measurements are significantly thinner than those of ultrasound pachymetry. The relationship of the pachymetry measurements obtained using the Orbscan II on keratoconic corneas with and without the 0.92 acoustic factor, as well as axial power measurements, compared to those obtained using the Pentacam HR and Galilei G2 is explored in chapter 8.

3.4.1 Pentacam HR
3.4.1.1 Technical Aspects of the Pentacam HR
The Pentacam HR (Oculus, Wetzlar, Germany) is a computerised tomography device (Figure 3-5A.) which utilises a 14mm mono-chromatic slit illumination system, composed of blue light emitting diodes (LEDs) (475 nm), a single rotating Scheimpflug camera and a static camera (Figure 3-5B.). The Scheimpflug principle allows for imaging with a wide depth of focus, allowing a planar object that is not parallel to the image plane to be in focus. The slit beam produced by the device creates an illuminated section which is imaged 12 to 50 times by the rotating Scheimpflug camera, corresponding to specific angles along the optical axis.

This system acquires radial images along the illuminated plane, allowing for the structures from the anterior surface of the cornea, up to the anterior surface of the crystalline lens to be imaged. The static camera is utilised to detect the pupil’s contour in order to compensate for eye movement during image acquisition (fixation drift). The Pentacam HR measures more than 25,000 data points in approximately 2 seconds.

To estimate the corneal power/shape, the Pentacam HR measures geometric elevations, which are then converted to power/curvature values in the form of axial (sagittal) power/curvature or instantaneous (tangential) power/curvature. Similar to the Orbscan II, measured parameters include corneal pachymetry, corneal keratometry, anterior and posterior elevation and Scheimpflug photography of the lens. The Pentacam HR also produces an objective method of assessing corneal clarity (corneal densitometry) determined by the amount of backscattered light. Reduced corneal clarity or “haze” increases the amount of backscattered light which is detected by the Pentacam HR and measured in grayscale units (GSU). GSU is a proprietary, relative scale ranging from 0-100, where 0 equates to minimum backscatter (highest transparency) and 100 to maximum backscatter (lowest transparency).
3.4.1.2 Utilisation of the Pentacam HR

To acquire the images utilising the Pentacam HR, the participant is asked to place their chin on the chin rest and forehead against the forehead bar (Figure 3-5E.). The height of the chin rest is altered to align the participant with the outer canthus marking and the table height is altered in order to ensure the participant is comfortable. Once in place, a blue illumination slit and red fixation light is visible to the participant. The participant is asked to maintain fixation on the red fixation light and to avoid blinking for the duration of image acquisition.

The Pentacam HR software directs the operator to align the device in the x, y and z axes using red on-screen arrows (Figure 3-5C. and D.). Following correct alignment by the operator, the Pentacam automatically starts the acquisition sequence. However, the acquisition sequence can be initiated manually by the operator when required, e.g. highly irregular corneas where the alignment system does not function.

The device was set to acquire 25 slit images of the anterior segment, taking approximately 2 seconds. The Pentacam HR provides a quality specification of “OK” if the scan is of acceptable quality.
Figure 3-5 The Pentacam HR (Oculus, Wetzlar, Germany)  
A. The Pentacam HR  
B. Scheimpflug camera, blue slit beam and static camera  
C. Improper alignment of the Pentacam HR and arrows directing operator to obtain proper x, y and z-axis alignment  
D. Proper alignment of the Pentacam HR  
E. The Pentacam HR in clinical use
The Pentacam HR and the above method of acquisition, were utilised in the investigations detailed in chapters 5, 8 and 9. The data obtained from the processed Scheimpflug images are presented by the device’s software in a quad-map format similar to the Orbscan II (Figure 3-6).

The following parameters obtained from the Pentacam HR were utilised as specified in chapters 5, 8 and 9: steep simulated keratometry ($K_{steep}$), flat simulated keratometry ($K_{flat}$), mean simulated keratometry ($K_{mean}$), maximum corneal power ($K_{max}$), obtained from axial power, central corneal thickness (CCT), thinnest corneal thickness (TCT), posterior elevation and corneal densitometry. Of note, the Pentacam has been shown to have good repeatability in both normal\textsuperscript{206} and keratoconic corneas.\textsuperscript{207} The repeatability and comparability of $K_{steep}$, $K_{flat}$, CCT and TCT on keratoconic corneas on the Pentacam HR in comparison to the same parameters in the Galilei G2 and Orbscan II is investigated in chapter 8.

![Refractive quad-map of the Pentacam HR (Oculus, Wetzlar, Germany) in a participant with moderate keratoconus, displaying: axial power, pachymetry, anterior elevation and posterior elevation maps](image)

**Figure 3-6** Refractive quad-map of the Pentacam HR (Oculus, Wetzlar, Germany) in a participant with moderate keratoconus, displaying: axial power, pachymetry, anterior elevation and posterior elevation maps
3.4.3 Galilei G2

3.4.3.1 Technical Aspects of the Galilei G2

The Galilei G2 computerised corneal tomographer (Ziemer Ophthalmology Co. Allmendstrasse, Switzerland) (Figure 3-5) combines dual rotating Scheimpflug cameras, a Placido disc, static CCD camera and a blue slit beam, produced by a blue LED (475nm). The dual Scheimpflug cameras are placed 180° apart in order to compensate for errors that may occur when acquisition is carried out at an oblique angle. As a result, the manufacturer suggests that the Galilei G2 provides greater accuracy in the measurement of pachymetry of the central and peripheral cornea, even if there are minor eye movements. While the Pentacam is a solely Scheimpflug-based system that derives keratometry data of the anterior surface solely from the geometry of the Scheimpflug images, the Galilei system uses an arc-step algorithm, similar to the Orbscan II and Medmont E-300, to reconstruct the corneal profile of the anterior surface as a series of arcs from the mires produced by the Placido rings.208

The Galilei G2 measures more than 122,000 data points per scan with an acquisition time of 1 to 2 seconds.198 The Scheimpflug images, allow for analysis of the anterior cornea, posterior cornea, anterior lens, and iris. The slope data obtained from the edges of the Placido ring images are converted to height data and height data determined from the Scheimpflug images are combined using a proprietary method to produce elevation maps.

3.4.3.2 Utilisation of the Galilei G2

Acquisition of images on the Galilei G2 is similar to other devices; the participant is asked to place their chin on the chin rest and forehead against the forehead bar (Figure 3-7E.). The height of the chin rest is altered to align the participant with the outer canthus marking and the table height altered to make the participant comfortable. Once in place, the participant is able to see the Placido disk consisting of the concentric red rings, the blue slit beam and a central red fixation target (Figure 3-7B.).

The participant is asked to maintain steady fixation on the red fixation target within the instrument and to avoid blinking for the duration of the scan. The operator centres the alignment cross with the four Purkinje images to ensure x and y-axis alignment (Figure 3-7C. and D.). Once x and y-axis alignment is achieved, the operator alters the distance from the corneal apex until the Scheimpflug image just touches the horizontal alignment line to achieve z-axis alignment (Figure 3-7C. and D.). Once correctly aligned, the operator manually initiates image acquisition. The Galilei breaks down the percentage quality of the image into four components: motion compensation, Placido, Scheimpflug, and motion distance. These components are then summarized as an overall quality score along with a reference minimum required percentage score.
Figure 3-7 The Galilei G2 (Ziemer Ophthalmology Co., Allmendstrasse, Switzerland) A. The Galilei G2 B. Dual Scheimpflug cameras, blue slit beam, static camera and Placido rings C. Improper alignment of the Galilei G2, arrows indicate the direction the device is to be moved to obtain proper alignment D. Proper alignment of the Galilei G2, arrows highlight the indicators of alignment, red crosshairs with mires (x and y-axis) and anterior edge of Scheimpflug image of the cornea and red horizontal indicator (z-axis) E. The Galilei G2 in clinical use
The Galilei G2 and aforementioned method of acquisition, was utilised in the investigation detailed in chapter 8. The data obtained from the processed Scheimpflug and Placido images are typically presented by the device’s software in a quad-map (Figure 3-8).

The following parameters obtained from the Galilei G2 were utilised as specified in chapter 8: steep simulated keratometry \( (K_{\text{steep}}) \) and flat simulated keratometry \( (K_{\text{flat}}) \), obtained from axial power, central corneal thickness (CCT), thinnest corneal thickness (TCT). The Galilei G2 has been shown to have good repeatability in both normal\(^{206}\) and keratoconic corneas.\(^{207}\) The repeatability and comparability of \( K_{\text{steep}} \), \( K_{\text{flat}} \), CCT and TCT on keratoconic corneas on the Galilei G2 to the same parameters on the Pentacam HR and Orbscan II is explored in chapter 8.

![Figure 3-8](image)

**Figure 3-8** Refractive quad-map of the Galilei G2 (Ziemer Ophthalmology Co., Allmendstrasse, Switzerland) in a participant with moderate keratoconus, displaying: axial power, pachymetry, anterior elevation and posterior elevation maps

### 3.4.4 Medmont E-300

#### 3.4.4.1 Technical Aspects of the Medmont E-300

The Medmont E-300 (Medmont Pty Ltd, Camberwell, Australia) is a Placido disk-based computerised corneal topographer (Figure 3-9A.), utilising 32 Placido rings, a central green ring (565nm) and 31 concentric red rings (660nm) in the shape of a cone (Figure 3-9B.). The CCD camera located at the centre of the cone captures images at a rate of 25 frames per second and the device measures 9,600 data points per scan. The Medmont E-300 utilises an arc-step reconstruction algorithm to analyse the mires of the Placido rings to extrapolate data regarding anterior corneal power/shape.\(^{209, 210}\)
3.4.4.2 Utilisation of the Medmont E-300

Acquisition of images on the Medmont E-300 begins in a similar fashion to other devices; the participant is asked to place their chin on the chin rest and forehead against the forehead bar. The height of the chin rest is altered to align the participant with the outer canthus marking and the table height is altered in order to make the participant comfortable. Once in place, the participant is able to see the Placido disk consisting of the concentric red rings and central green fixation ring (Figure 3-9B.).

The participant is asked to maintain steady fixation on the green fixation ring within the instrument and to avoid blinking for the duration of the scan. The operator centres the alignment cross with the centre of the mires of the Placido disk to ensure x and y-axis alignment. Once x and y-axis alignment is achieved, the device instructs the operator to move the device forward or backward to achieve z-axis alignment and focused images (Figure 3-9C. and D.).

Once correctly aligned, the instrument automatically captures images. Each image captured is assigned a score out of 100 based on centration, focus (distance from eye) and steadiness of fixation (eye movement). A score > 75 was considered acceptable and this criterion was used when selecting scans for data collection.
Figure 3-9 The Medmont E-300 (Medmont Pty Ltd, Camberwell, Australia) A. The Medmont E-300 B. Placido rings of the Medmont E-300, arrows indicate the direction the device is to be moved to obtain proper alignment C. Improper alignment of the Medmont E-300, arrows indicate the direction the device is to be moved to obtain proper alignment D. Proper alignment of the Medmont E-300, arrow highlights the indicators of alignment, green cross-hairs centred on centre of Placido ring mires (x and y-axis alignment) and red horizontal line touching horizontal arm of the green cross-hairs (z-axis alignment) and automatic image acquisition.
The Medmont E-300 and the aforementioned method of acquisition, was utilised in the investigation detailed in chapter 7. The data obtained from the processed Placido disk images of the Medmont E-300 are typically presented by the device’s software in a single-map format, in contrast to the tomography devices discussed above (Figure 3-10). Additionally, the Medmont E-300 is solely a Placido disk based corneal topographer hence only assessment of the anterior corneal surface is possible and the device does not assess pachymetry or posterior corneal elevation. Thus only axial or tangential power maps and anterior elevation maps are produced. The following parameters obtained from the Medmont E-300 were utilised in chapter 7: steep simulated keratometry ($K_{\text{steep}}$), flat simulated keratometry ($K_{\text{flat}}$), maximum corneal power ($K_{\text{max}}$), the inferior-superior dioptric asymmetry ($I-S$)$^{211,212}$, surface asymmetry index (SAI)$^{213,214}$ and surface regularity index (SRI)$^{215,216}$ all obtained from the axial power map. The Medmont E-300 has been shown to be highly repeatable on test surfaces$^{217}$ and normal corneas,$^{218}$ however, the repeatability has not previously been assessed on keratoconic corneas, which is addressed in chapter 7.

![Figure 3-10](image.png)

**Figure 3-10** Axial power map of the Medmont E-300 (Medmont Pty Ltd, Camberwell, Australia) in a participant with moderate keratoconus: axial power, simulated keratometry, inferior-superior dioptric asymmetry ($I-S$ index), surface asymmetry index (SAI) and surface regularity index (SRI)
3.4.5 Diagnosis of Keratoconus

The tomographic diagnostic criteria for keratoconus are still the focus of much debate. The majority of developments in the diagnosis of keratoconus have focused on further analysing corneal tomography, such as assessment of spatial distribution of corneal thickness and Fourier analysis of keratometric data. In chapters 5, 8 and 9 of this thesis the criteria for a diagnosis of keratoconus was primarily based on computerised corneal tomographic features obtained from the Pentacam HR:

Primary features:
- Increased posterior elevation above the BFS (>40µm)
- Increased corneal power/curvature (mean simulated keratometry >45.0D)
- Asymmetric steeping of the anterior surface (I-S value >1.20D)

Secondary features:
- Reduction in central corneal thickness (CCT ≤500µm)
- Reduced best spectacle corrected visual acuity
- Refractive cylinder >2.00D

All of the primary features had to be present for a diagnosis to be confirmed whereas the secondary features were not essential and were intended to mainly assist in borderline cases.

In chapters 4, 6 and 7, the diagnosis of keratoconus was made by community optometrists and the criteria for diagnosis were made at their discretion. However, their criteria included: increased corneal curvature, decreased corneal thickness, reduced best spectacle corrected visual acuity, increased refractive cylinder. Most optometrists in NZ do not have access to tomographic devices hence posterior elevation was not part of the diagnostic criteria.

3.5 Anterior Segment Optical Coherence Tomography

Optical coherence tomography (OCT) is utilised to image sub-surface details of biological tissue. OCT is based on the principle of low-coherence interferometry, whereby a light source with a broad bandwidth is split into two beams by a beam splitter, a sample beam that contains the object of interest and a reference beam from a mirror. Differences in the path length of the reflected sample and reference beams produce interference patterns, providing information on the optical properties of sub-surface structures, hence alluding to the composition and thickness of said structures.
3.5.1 Heidelberg Spectralis

3.5.1.1 Technical Aspects of the Heidelberg Spectralis Anterior Segment Module

The Heidelberg Spectralis (Heidelberg Engineering GmbH, Heidelberg, Germany) (Figure 3-11A.) is a spectral domain OCT, the beam of which is produced by a super luminescence diode (average wavelength 870nm (infrared)) (Figure 3-11B.). Originally designed to image the retina, the Heidelberg Spectralis can be modified with a specialised anterior segment lens and software to image the cornea. In the high resolution setting, the Heidelberg Spectralis has a lateral resolution of 5 μm/pixel and image acquisition rate of 5 Hz to 9 Hz.

3.5.1.2 Utilisation of the Heidelberg Spectralis Anterior Segment Module

Acquisition of OCT images utilising the Heidelberg Spectralis Anterior Segment Module (HSASM) is similar to the other devices mentioned; the participant is asked to place their chin on the chin rest and forehead against the forehead bar (Figure 3-11E.). The height of the chin rest is altered to align the participant with the outer canthus marking and the table height altered to make the participant comfortable. Once in position, the participant is able to see a central red fixation target. The participant is asked to maintain steady fixation on the red fixation target within the instrument and that they should blink as usual but keep their eyes open wide between blinks for the duration of the scan. The active eye tracking (ART) was set to 25 (out of a possible 60) in order to obtain high-quality images of the central cornea. The higher the ART setting the longer the acquisition time, hence ART was set at 25 instead of 60 to produce images of adequate quality within a reasonably short acquisition time. The “small” scan setting consists of 11 parallel scans, each 8mm in length.

The scan field is manually aligned with the geometric centre of the cornea by the operator to obtain x and y-axis alignment (Figure 3-7C. and D.). Once x and y-axis alignment is achieved, the operator alters the distance from the corneal apex until the OCT image is within the blue alignment boxes to achieve z-axis alignment (Figure 3-11C. and D.). Once correctly aligned, the operator manually initiates image acquisition. The operator maintains alignment manually while images are acquired automatically by the device software.
The Heidelberg Spectralis Anterior Segment Module is utilised in chapter 9. The calliper tool in-built into the device software was utilised to determine the depth of the post-operative stromal demarcation line observed following corneal collagen cross-linking. The depth of the demarcation was measured at the geometric centre of the cornea, from Bowman’s layer to the posterior edge of
the demarcation line and was represented as a percentage of the corneal thickness measured from Bowman’s membrane to the posterior edge of the corneal endothelium in the same plane.

3.6 Corneal Biomechanics

As mentioned in chapter 2, keratoconus is believed to the result of biomechanical compromise to the cornea. Previous evidence of this phenomenon has previously been limited to ex vivo analysis. However, recent technological advances have produced devices, such as the CorVis ST, designed to assess corneal biomechanical properties in vivo.

3.6.1 CorVis ST

3.6.1.1 Technical Aspects of the CorVis ST

The CorVis ST (CST) (Oculus, Wetzlar, Germany) (Figure 3-12A.) is a device which combines an air-pulse analogous to a non-contact tonometer with a high-speed Scheimpflug camera in order to assess the biomechanical properties of the cornea in vivo. The CST emits a collimated air-pulse 3.05mm in diameter, with a force of 60 mm Hg at the nozzle. The air-pulse indents the cornea, deforming it inward into a concave configuration prior to returning to its initial shape once the air-pulse is ceased. Cross-sectional images of the cornea during and after application of the air-pulse are recorded as a video sequence using the high-speed Scheimpflug camera (4330 frames/sec). This sequence is obtained in less than 32ms, over which time the cornea undergoes first inward applanation (flattening), followed by deformation to maximum concavity, and finally, second outward applanation as the air-pulse ceases and the cornea returns to its original shape. Individual frames within the video sequence are analysed by the in-built CST software to produce a number of unique measurements of physical parameters, including deformation amplitude (DA) (Figure 3-12E.).

3.6.1.2 Utilisation of the CorVis ST

Corneal biomechanical properties were assessed using the CorVis ST as follows. The participant was instructed to place their chin on the chin rest and forehead against the forehead bar (Figure 3-12D.) The participant’s cornea was centred using the red alignment arrows provided on the machine’s display in order to obtain x, y and z-axis alignment, analogous to the Pentacam HR, the measurement is automatically initiated by the device when properly aligned but can also be manually initiated (Figure 3-12B. and C.). The investigations detailed in chapters 5 and 9 focused on the maximum corneal deformation under the influence of the air-pulse, the deformation amplitude, as this parameter has previously been shown to be significantly increased in subjects with keratoconus.
Figure 3-12 The CorVis ST (Oculus, Wetzlar, Germany): A. The CorVis ST B. Improper alignment of the CorVis ST, the red arrow instructs the operator how to alter the position of the device to obtain proper x, y and z-axis alignment C. Proper alignment of the CorVis ST D. The CorVis ST in clinical use E. Scheimpflug images of the cornea under the influence of the air-pulse emitted from the CorVis ST

3.7 In Vivo Confocal Microscopy of the Cornea

In vivo confocal microscopy (IVCM) devices have been commercially available for over two decades and have been utilised to assess the microstructure of the cornea in health and disease. The microstructural changes that occur in keratoconus have been outlined in chapter 2.

3.7.1 Heidelberg Retina Tomograph II, Rostock Corneal Module

3.7.1.1 Technical Aspects of the Heidelberg Retina Tomograph II, Rostock Corneal Module

The Heidelberg Retina Tomograph II Rostock Corneal Module (HRTII) (Heidelberg Engineering, GmbH, Germany) (Figure 3-13A.) is a laser scanning in vivo confocal microscope, utilising a coherent Helium Neon diode laser (670nm). The laser is deflected in two perpendicular directions by two
scanning mirrors and is scanned sequentially over each point in the examined area.\textsuperscript{225} The beams are scanned horizontally and vertically by the mirrors to produce horizontal and vertical scan fields. The reflected light is de-scanned by the same scanning mirrors.\textsuperscript{225} The reflected light is deflected toward a photo diode and digitised to form an image.\textsuperscript{225} The HRT also has a coupled CCD camera lateral to the IVCM, allowing the operator to monitor the position of the HRTII relative to the cornea. The applanating cap (tomocap) of the HRTII must applanate the cornea in order to image the corneal microstructure. With the 60x objective water immersion lens with a numerical aperture of 0.9 (Olympus, Tokyo, Japan) the IVCM images that are produced are 400 x 400 μm in dimension with a digital image size 384 x 384 pixels (1.04 μm/pixel), a lateral resolution of 2μm and optical section thickness of 4μm. The HRTII enables imaging of all the major cell types of the cornea including the: epithelium, sub-basal nerve plexus, keratocytes and endothelium (Figure 3-16).

3.7.1.2 Utilisation of the Heidelberg Retina Tomograph II, Rostock Corneal Module
To capture images using the HRT II, both eyes were anaesthetised 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) was placed between the lens and a sterile, disposable applanating lens cap (tomocap) (Figure 3-13B. & C.). The participant was asked to place their chin on the chin rest and forehead against the forehead bar (Figure 3-13E.). The height of the chin rest was altered to align the participant with the outer canthus marking and the table height was altered in order to make the participant comfortable.

The participant was asked to fixate on the laser visible inside the device, in order to assess the central cornea, and the tomocap was manually advanced toward the cornea until applanation was achieved without compression (Figure 3-13E.) The full thickness of the central cornea was examined using the ‘section’ mode i.e. single images are manually acquired by the observer at any desired corneal depth and the focal position (in μm) is automatically displayed on the computer screen and recorded for each image saved (Figure 3-13D.).
Figure 3-13 The Heidelberg Retina Tomograph II Rostock Corneal Module (HRTII) (Heidelberg Engineering, GmBH, Germany) A. The HRTII B. Application of Viscotears onto objective lens C. Viscotears visible between objective lens and applanating lens cap D. View of lateral CCD camera, focus depth and image of keratocytes being captured E. The HRTII in clinical use
For the investigations detailed in chapters 5 and 9, three images of each of the following layers were collected; the central basal corneal epithelium, sub-basal corneal nerve plexus, anterior and posterior keratocytes. The basal epithelium was defined as the first three clear, compression free images of the epithelium anterior to Bowman’s layer. Anterior keratocytes were defined as the first three clear, compression free images of the keratocytes posterior to Bowman’s layer. Posterior keratocytes were defined as the first three clear, compression free images of keratocytes anterior to Descemet’s membrane.

Quantitative analysis was performed by a single examiner (AG) using the Image J software (U. S. National Institutes of Health, Bethesda, Maryland, USA, available in the public domain at http://rsb.info.nih.gov/ij/). The manual cell counter function was utilised to perform cell counts, subsequently converted to cell densities, for the corneal basal epithelium, anterior and posterior keratocyte images. Only epithelial cells or keratocyte nuclei within the clearest 192 x 192 pixel (200 x 200 µm) region of an image were counted. Cell counts utilised the “L” method, whereby cell borders/cell nuclei crossing the left and inferior border were counted whereas those crossing the right and superior borders were omitted (Figure 3-14A., C. and D.).

The Neuron J plugin for Image J (developed by Associate Professor Erik Meijering, Biomedical Imaging Group Rotterdam of the Erasmus University Medical Centre, Rotterdam, and downloadable from the public domain at http://www.imagescience.org/meijering/software/neuronj/) was utilised to produce semi-automated calibrated tracings of the sub-basal nerve plexus. All nerves contained within the entire 400 x 400 µm image were traced and subsequently converted to a nerve density (Figure 3-14B.)

All images were anonymised and randomised prior to analysis by an independent observer (IC). The mean of the quantitative analysis of three images was utilised to obtain the central corneal basal epithelial cell density (cells/mm^2), sub-basal corneal nerve density (mm/mm^2), anterior and posterior keratocyte density (cells/mm^2).
Cell counts or nerve tracings conducted on *in vivo* confocal microscopy images obtained with the Heidelberg Retina Tomograph II Rostock Corneal Module (Heidelberg Engineering, GmBH, Germany). 

A. Corneal epithelium  
B. Corneal sub-basal nerve plexus  
C. Anterior keratocytes  
D. Posterior keratocytes

3.7.2 Confoscan 4

3.7.2.1 Technical Aspects of the Confoscan 4

The Confoscan 4 (CS4) (Nidek Technologies, Gamagori, Japan) (Figure 3-15A.) is a slit scanning *in vivo* 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.\(^{225}\) With the 40x objective lens, the lateral resolution achieved is 0.6µm/pixel, with an inspected field of 460 x 345 µm, an image size of 768 x 576 pixels and section thickness of up to 26 µm.\(^{227}\)
3.7.2.2 Utilisation of the Confoscan 4

Both eyes were anaesthetised utilising topical 0.4% benoxinate hydrochloride (Chauvin Pharmaceuticals, Surrey, UK). The participant was requested to place their chin on the chin rest and head against the forehead bar (Figure 3-15B.). When in place, the participant was instructed to fixate on an internal fixation target to enable examination of the central cornea. The exposed tip of the objective lens was covered with a viscous contact solution Carbomer 980 (Viscotears 0.2%; Novartis, North Ryde, NSW, Australia). A semi-automated acquisition technique was utilised whereby, the objective 40x gel immersion lens is advanced manually by the operator until the gel was in contact with the surface of the cornea. The operator then adjusted the position of the lens until the microscope was centred on the corneal stroma and initiated automated alignment and scanning.

Automatic scanning obtains multiple single section images across the entire thickness of the central cornea entailing all the major cell types of the cornea: epithelium, sub-basal nerve plexus, keratocytes and endothelium. Full-thickness scans are acquired at 25 frames/second, with 350 images obtained per scan and each image acquired at 5 μm depth intervals. The images are then transferred and stored on the NAVIS application (Nidek Technologies, Gamagori, Japan).

The axial resolution of the CS4 produces sections up to 26 µm thick, significantly thicker than that of the HRTII (4 µm), hence images of the epithelium, sub-basal nerve plexus and keratocytes are of poorer quality than those produced by the HRTII (Figure 3-16). However, the CS4 provides superior quality images of the endothelium thus the CS4 was utilised to assess corneal endothelial cell density. Quantitative analysis was performed by a single examiner (AG) using the Image J software (U. S. National Institutes of Health, Bethesda, Maryland, USA). The manual cell counter function was utilised to determine cell counts, subsequently converted to cell densities.

As with the HRT II image analysis, only cells within the clearest 200 x 200 μm (333 x 333 pixels) region of an image were counted. Cell counts utilised the “L” method, whereby cell borders/cell nuclei crossing the left and inferior borders were counted, whereas those crossing the right and superior borders were omitted (Figure 13-15D.). All images were anonymised and randomised prior to analysis by an independent examiner (IC).
Figure 3-15 The Confoscan 4 (Nidek Technologies, Gamagori, Japan) A. The Confoscan 4 B. & C. Application of Viscotears onto the objective lens D. Cell count conducted on *in vivo* confocal microscopy image of the corneal endothelium obtained with the Confoscan 4 E. The Confoscan 4 in clinical use
Figure 3-16 *In vivo* confocal microscopy images of the central cornea obtained with the Confoscan 4 (A, C, E, G and I) and the Heidelberg Retina Tomograph II Rostock Corneal Module (B, D, F, H and J)
Section 2:

The Epidemiology, Demographics, Distribution and Basic Clinical Characteristics of Subjects with Keratoconus in New Zealand – The Aotearoa Research into Keratoconus (ARK) Study: Part I

Chapter 4
Chapter 4:

The Epidemiology, Demographics, Distribution and Basic Clinical Characteristics of Subjects with Keratoconus in New Zealand – The Aotearoa Research into Keratoconus (ARK) Study: Part I
4.1 Introduction

As touched upon in chapter 2, keratoconus is relatively common with estimates of prevalence varying from 6.8–2300/100,000.\textsuperscript{1, 4, 7, 9–11} This variation may partly be due to a genetic predisposition but other factors include; diagnostic criteria used and the retrospective, case-based study design of most epidemiological studies.\textsuperscript{1, 4, 7, 9} Unfortunately, most epidemiological studies of keratoconus also occur in tertiary centres\textsuperscript{1, 4, 7, 9} where more advanced cases are generally managed, paradoxically these may constitute the minority of cases.

Advances in rigid gas permeable (RGP) lens technologies have made it possible for most cases of keratoconus to be managed by community optometrists, thus estimates obtained from tertiary centres probably underestimate the true prevalence of keratoconus. Significantly, a recent investigation of those with keratoconus attending a tertiary, hospital-based, sub-specialty cornea service in Auckland, New Zealand/Aotearoa (NZ) revealed high proportions of patients with severe keratoconus (58.3%).\textsuperscript{39}

Indications for corneal transplantation in NZ suggest that transplantation is performed for keratoconus more commonly than in other countries within the Organisation for Economic Co-operation and Development (OECD); 41.1 to 45.6% (NZ),\textsuperscript{40, 41} 31.0% (Australia),\textsuperscript{42} 22.5% (UK),\textsuperscript{45} and 16% (USA).\textsuperscript{46} These data and clinical experience have raised the suspicion of a high prevalence of keratoconus in NZ, however, the only large scale, population-based investigation of the epidemiology of keratoconus in NZ was conducted in 1978.\textsuperscript{12} This study concluded that keratoconus occurred in 1/2000 of the population and was equally present in Māori and European peoples, however, this study was localised to a single small region, Hawkes Bay, in NZ.\textsuperscript{12}

Therefore, a better, contemporary, understanding of the epidemiology, demographic and clinical characteristics of keratoconus in NZ would be beneficial, particularly with respect to: refining the disease profile to identify at risk individuals/populations, optimising long term management of the affected population, and appropriately providing access to modalities such as contact lenses (CLs), corneal collagen cross-linking (CXL) and corneal transplantation.\textsuperscript{228}

New Zealand (Aotearoa) is an island nation in the South Pacific, slightly larger (268,000 Km\textsuperscript{2}/103,500 miles\textsuperscript{2}) than the United Kingdom, with a population of 4.47 million located within two major Islands. It has a highly trained, widely distributed, eye health work force with approximately 120 ophthalmologists and 627 optometrists.
Aims

The aims of the ARK Study: Part I was to:

1) Investigate the epidemiology and geographic distribution of keratoconus in NZ
2) Characterise the demographics of individuals with keratoconus
3) Determine if any ethnicities are over-represented in the keratoconus population
4) Characterise the clinical characteristics of individuals with keratoconus managed by optometrists

4.2 Methods

4.2.1 Study Protocol and Design

A two-year prospective, longitudinal, observational study, focused on individuals with keratoconus managed by optometrists in NZ. A digital web-based survey protocol (Appendix B) was completed by all participating optometrists for every subject with keratoconus (in at least one eye) that attended a consultation between 30th January 2014 and 30th January 2016.

4.2.2 Outcome Measures

The following parameters were requested for each subject:

- Date of birth
- Gender
- Self-reported ethnicity
- Whether the subject had a new or prior diagnosis of keratoconus

The following parameters were requested/calculated for all eyes:

- Uncorrected vision (UCVA)
- Best corrected visual acuity (BCVA)
- Type of refractive correction used to obtain BCVA
- Measurements of corneal curvature/power; steep keratometry ($K_{steep}$), flat keratometry ($K_{flat}$) and mean keratometry $K_{mean}$ – obtained from computerized corneal topography or manual/automated keratometry
- Any corneal surgical procedures (and in which eye) including corneal transplantation and CXL
- Any other ocular pathology (and in which eye) including active or resolved acute cornea hydrops
- Disease severity was categorised using the Amsler-Krumeich grading system for keratoconus, based on keratometry alone\cite{229}
- The two major islands (North and South) of New Zealand are divided into 16 regions. The distribution of cases of keratoconus was determined by the region in which the participating optometrists practiced
4.2.3 Participants

The participants of this study were optometrists in the wider community across NZ who provided data on subjects with keratoconus that attended a consultation. Regular recruitment announcements were sent out to optometrists via the newsletters distributed by the New Zealand Association of Optometrists (NZAO) and Cornea and Contact Lens Society of New Zealand (CCLS) (Appendix C) in order to encourage the highest possible proportion of optometrists to participate. Additionally, similar to the ARK Study: Part IIA (chapter 5), this study was included in the author’s conference presentations on the ARK project at the NZAO and CCLS annual national conferences in 2013 and 2014 to further encourage optometrists in NZ to participate. Reminder emails were sent out to participating optometrists every two months over the entirety of the study period to encourage them to continue to complete the survey protocol for all subjects with keratoconus that presented for a consultation. These emails were intended to ensure that the majority of subjects that underwent a consultation were captured by the study.

All data on subjects received from participating optometrists was anonymised, with no personal details of the subjects provided to the investigators. This investigation adhered to the tenets of the Declaration of Helsinki and ethical approval to carry out the study was obtained from the University of Auckland Human Participant Ethics committee (UoAHPEC), approval number: 010547.

Inclusion Criteria

- All registered practicing optometrists in NZ were eligible and were encouraged to participate in this study
- Participating optometrists were requested to complete the survey protocol for all subjects with manifest keratoconus in at least one eye that presented for a consultation over the two-year study period. As stated in chapter 3, the diagnosis of keratoconus was made by participating optometrist, on the basis of examination, likely utilising a combination of keratometry, slit-lamp biomicroscopy and refraction

Exclusion Criteria

- There were no exclusion criteria per se and all subjects with keratoconus that underwent a consultation were eligible. However, as previously stated, optometrists were requested to report on other ocular features and in which eye they occurred including; previous/current episode of corneal hydrops, previous ocular surgery or trauma, and any other ocular pathology. Eyes that had any of the above features were excluded from the eye-specific statistical analyses
**Statistical Analysis**

The distribution of cases of keratoconus across NZ was compared qualitatively to the distribution of the overall NZ population via data obtained in the 2013 Census. To determine if the sample was representative of the entire population of individuals with keratoconus, the distribution of optometrists participating in the ARK study was compared to that of all practicing optometrists in NZ. The chi square test and adjusted standardised residuals post-hoc analysis was used to compare the relative proportion of participating optometrists in each region to the proportion of practicing optometrists in that region. Ethnicities were self-reported by the subjects and subsequently grouped in the same manner as the 2013 NZ Census; European, Māori, Pacific Peoples, Asian, Middle Eastern/Latin American/African. The ethnicity distribution of the ARK database was compared to that of the population captured by the 2013 NZ Census.

Analysis of differences in eye specific variables by ethnicity was carried out using all eligible eyes and available data. The chi square test and adjusted standardised residuals post-hoc analysis was used for categorical variables (Amsler-Krumeich grading) and the one-way ANOVA with Tukey post-hoc analysis for continuous variables (UCVA, BCVA and $K_{\text{mean}}$). Eyes that had undergone any surgery or had other pathology were excluded from further analyses.

All analyses were performed using SPSS 22.0 (Chicago, IL, USA). A p-value of <0.05 was considered significant throughout.
4.3 Results

4.3.1 Epidemiology and Demographics

Data from 1,869 subjects with keratoconus was received from 66/627 (10.5\%) optometrists practising in 11/16 regions of NZ. The mean age of the subjects with keratoconus was 41.0 ± 15.7 years, 1055 (56.4\%) male, 238 (12.7\%) newly diagnosed and 1631 (87.3\%) had a prior diagnosis of keratoconus.

The majority of subjects with keratoconus (93\%) reside in 4/16 regions of NZ (Auckland (41.6\%), Waikato (21.3\%), Wellington (16.8\%) and Bay of Plenty (13.3\%)). This is disproportionate relative to the overall population proportions residing within the same regions (Figure 4-1).

The comparison between the distribution of optometrists participating in the ARK study and all practicing optometrists in NZ revealed that of the 11 regions covered, only Wellington had a disproportionately high proportion of participating optometrists; 21.2\% of all participants while 11.6\% of all practicing optometrists were based in Wellington (p=0.026).

There was a greater proportion of Māori and Pacific Peoples in the study population (22.7\% and 15.5\%, respectively) compared to the general population (13.5\% and 6.6\%, respectively), while all other ethnicities were under-represented (Figure 4-2 A. and B.).
Figure 4-1 Comparison of percentage distribution of subjects with keratoconus (N=1869) and overall population across New Zealand, by region. All values are proportion of subjects included in the ARK study vs. proportion of overall population resident in that region.
**4.3.2 Clinical Characteristics**

Clinical parameter data were ineligible in 18.5% of eyes of 28.0% of subjects, therefore analyses were performed only on eyes with available data (n=2439 (65.3%) eyes of 1573 (84.2%) subjects). The following were ineligible for further analysis; 435 (11.6%) eyes of 320 (17.1%) subjects had undergone corneal transplantation, 89 (2.4%) eyes of 69 (3.7%) subjects had been treated by CXL, 35 (0.9%) eyes of 31 (1.7%) subjects had a history of acute corneal hydrops, 136 (3.6%) eyes of 102 (5.5%) subjects had other ocular pathology or underwent surgery.

The mean UCVA, BCVA, $K_{\text{steep}}$, $K_{\text{flat}}$ and $K_{\text{mean}}$ for all analysed eyes was; $0.8 \pm 0.5$ LogMAR (6/42), $0.2 \pm 0.1$ LogMAR (6/9), $51.0 \pm 6.5D$, $47.1 \pm 5.2D$ and $49.0 \pm 5.7D$, respectively. The majority of eyes required a RGP CL (64.3%) to achieve their BCVA, the most common type was a corneal lens (34.2%) (Figure 4-3A.). The majority of eyes were of mild-moderate disease severity, 52.5% stage I and 29.6% stage II on the Amsler-Krumeich scale (Figure 4-3B.).
Comparison of disease severity between ethnicities is highlighted in table 4-1. Eyes of subjects of Māori and Pacific Peoples had the highest average $K_{\text{mean}}$ with no significant difference between these two ethnicities. However, the average $K_{\text{mean}}$ of eyes of both Māori and Pacific Peoples was significantly higher than those of Europeans ($p < 0.03$). Māori and Pacific Peoples also had the greatest proportions of eyes graded stage IV on the Amsler-Krumeich scale ($p < 0.001$) and the lowest proportions of eyes with less severe disease i.e. graded stage I ($p < 0.001$) (Table 4-1). However, there was no difference in UCVA and BCVA between eyes of subjects of all ethnicities ($p > 0.070$ and $p > 0.400$, for UCVA and BCVA, respectively).

**Table 4-1** Comparison of disease severity between ethnicities using all eyes with available data for $K_{\text{mean}}$, Amsler-Krumeich grade based on $K_{\text{mean}}$ alone, uncorrected vision (UCVA) and best corrected visual acuity (BCVA). Values are mean ± standard deviation or percentage of eyes

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>$K_{\text{mean}}$ (D)</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>UCVA (LogMAR)</th>
<th>BCVA (LogMAR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>European</td>
<td>48.4 ± 4.8*</td>
<td>55.8</td>
<td>31.0</td>
<td>5.9</td>
<td>7.4</td>
<td>0.9 ± 0.5</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>Māori</td>
<td>49.4 ± 5.7*</td>
<td>48.8†</td>
<td>29.0</td>
<td>6.9</td>
<td>15.3†</td>
<td>0.9 ± 0.5</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>Pacific Peoples</td>
<td>50.8 ± 7.8*</td>
<td>45.7†</td>
<td>26.7</td>
<td>5.7</td>
<td>21.9†</td>
<td>0.8 ± 0.5</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>Asian</td>
<td>49.1 ± 5.5</td>
<td>51.6</td>
<td>29.4</td>
<td>7.2</td>
<td>11.8</td>
<td>0.8 ± 0.5</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>Middle Eastern/Latin</td>
<td>48.7 ± 6.5</td>
<td>67.7</td>
<td>22.6</td>
<td>0.0</td>
<td>9.7</td>
<td>0.7 ± 0.4</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>American/ African</td>
<td>46.9 ± 3.8</td>
<td>66.7</td>
<td>26.7</td>
<td>0.0</td>
<td>6.7</td>
<td>0.7 ± 0.7</td>
<td>0.2 ± 0.1</td>
</tr>
</tbody>
</table>

*Significantly different mean on post-hoc analysis $p < 0.05$

† Significantly different proportion on post-hoc analysis $p < 0.001$
4.4 Discussion

New Zealand (Aotearoa) is located in the southwest Pacific, with a large land mass relative to a small population of approximately 4.5 million. Due to NZ’s geographical location, it has the largest populations of Māori and Pacific Peoples world-wide. There has been a long-held clinical impression that keratoconus is relatively common in NZ, particularly among the Māori and Pacific Peoples.

Although keratoconus, as an indication for corneal transplantation in NZ, is higher than elsewhere, the suspected ethnic predilection could not be fully explored due to limitations of available data.\textsuperscript{40, 41} The higher transplantation rates for keratoconus in NZ may also reflect more severe or rapidly progressive disease in local populations.\textsuperscript{40, 41, 189} Indeed, it has been shown that there is variation in the rate of progression between ethnicities,\textsuperscript{230} which may lead to differences in disease severity. Interestingly, the current study also suggests that Māori and Pacific Peoples have greater disease severity compared to other ethnicities as indicated by a significantly higher mean $K_{\text{mean}}$, the lowest proportions of mild disease (stage I) and highest proportions of severe disease (stage IV) on the Amsler-Krumeich scale, based on keratometry alone.

Investigations of the potential ethnic predilection of keratoconus for individuals of Māori and Pacific Peoples descent has already been explored in chapter 3 but is revisited here to provide context for the results of ARK Part I. The previously mentioned study by Jordan et al.,\textsuperscript{39} demonstrated higher proportions of Māori and Pacific Peoples, and lower proportions of Europeans and Asians, with keratoconus attending a hospital-based cornea service, compared to the total population of patients attending hospital services in Auckland. However, this study was limited to a tertiary eye centre where patients with more severe disease are generally treated, and thus may have been biased toward greater proportions of Māori and Pacific Peoples, who, as our study suggests have more severe keratoconus. Owens et al.,\textsuperscript{188} screened high school age children in NZ using corneal topography and noted up to 19% exhibited early keratoconus with Māori/Pacific Peoples twice as likely as European students to have suspicious corneal topography. However, just two specifically chosen schools in the Waikato region were screened, limiting the study’s wider applicability.\textsuperscript{188}

In contrast, Sabiston\textsuperscript{12} identified no ethnic predisposition in a relatively small population in the Hawkes Bay region and a nation-wide, patient-based, questionnaire survey of keratoconus also failed to identify an ethnic predisposition.\textsuperscript{187} This latter study\textsuperscript{187} like the current study compared ethnicity distribution of the keratoconus cohort to the overall population using contemporary census data. Nonetheless, the results of the current study bear greater similarity to the previous NZ studies of Jordan et al.,\textsuperscript{39} and Owens et al.,\textsuperscript{188} that highlighted an ethnic predilection, wherein both Māori and Pacific Peoples are over-represented compared to the overall population, while European and Asian ethnicities are under-represented.
The ARK study was carried out nation-wide and 11/16 NZ regions were represented by participating optometrists. The remaining five regions had a comparable population-based proportion of practicing optometrists. However, these regions are significantly less populated, representing only 9.8% of the total NZ population. Thus the ARK study addressed >90% of the NZ population. Additionally, in 4 of these 5 regions, the population was >85% European and <2.3% Pacific Peoples and since this study suggests that keratoconus is significantly more common in Pacific Peoples compared to Europeans, it is possible that these regions also contained fewer subjects with keratoconus. Thus the authors believe the results of this study contain a representative sample of keratoconus in NZ.

Keratoconus may be the result of both genetic predisposition and environmental factors such as eye rubbing (the two-hit hypothesis) and NZ has amongst the highest reported rates of asthma, eczema, and hay fever in the world, well-established associations of keratoconus. With a small, relatively isolated, population a genetic predisposition to keratoconus and atopic disease may be perpetuated, possibly resulting in a higher prevalence of keratoconus. Interestingly, asthma and eczema have been reported to be more prevalent in Māori and Pacific Peoples children than in European children in NZ, which may contribute to an increased risk of developing keratoconus.

Limitations of our study preclude the calculation of a meaningful estimate of the prevalence of keratoconus. Most subjects with keratoconus are under primary care to some extent as optometrists and ophthalmologists in NZ share care of many patients, however, data from those solely under tertiary care are absent. We also observe a potential self-selection bias as only optometrists who agreed to participate in the ARK study provided data, and some may have a special interest in keratoconus, and although this sample is a representative distribution of optometrists in NZ, the overall nationwide participation was 10.5%. A similar self-selection bias exists for patients; it is unlikely that all patients with keratoconus attend optometrists regularly or would have had a consultation in the study period. Thus, while we believe the large sample size is representative, it certainly does not contain all those with keratoconus in NZ during the study period. However, data regarding whether a patient had a new or prior diagnosis may provide some indication of relative incidence; approximately 1/10 subjects reviewed over the two year period exhibiting a “new” diagnosis of keratoconus.

A selection bias in the data on disease severity is also likely as data on all parameters was not available for all eyes due to technical or other limitations, which may have skewed the data toward lower disease severity as data from these eyes would be least difficult to obtain. Interpretations of the K-readings should be considered carefully as several different devices were utilised to obtain the...
data. There is also a limitation in the ethnicity-specific analyses of keratoconus severity which were carried out using all eligible eyes with available data; when data from only one eye is available due to the other eye being ineligible for analysis or having no data, the severity of the other eye is unknown, again potentially biasing the results toward lower disease severity. However, averaging the data from both eyes would have the potential to create a similar bias as keratoconus is known to be asymmetric. Therefore carrying out subject-specific analyses using all eligible eyes may better represent the average disease severity of each ethnic group.

The clinical characteristics of the ARK cohort can be compared to the baseline results of landmark international keratoconus studies such as the Collaborative Longitudinal Evaluation of Keratoconus (CLEK) and the Dundee University Scottish Keratoconus Study (DUSKS), carried out in the USA and UK, respectively. The mean age of subjects at baseline in CLEK (39.3 ± 10.9 years) was comparable to ARK Part I (41.0 ± 15.7 years) while DUSKS (30.9 ± 10.4 years) had a considerably younger mean age. The mean $K_{\text{flat}}$ was slightly higher in CLEK (49.5 ± 6.0D) than ARK Part I (47.1 ± 5.2D), while $K_{\text{flat}}$ was considerably higher in DUSKS (51.7 ± 5.4D). RGP CL use was similar in ARK Part I (64.3% of eyes) and CLEK (69.2% of eyes), (both optometry-based studies) but surprisingly only 9% of subjects in the hospital-based DUSKS wore CLs. Perhaps unsurprisingly the ARK Part I results appear to be more comparable to the CLEK, likely due to data acquisition at a primary care level in both studies, while DUSKS recruited patients referred to tertiary care. However, CLEK only reported $K_{\text{flat}}$ for the worse eye, thus its similarity to our study suggests the disease severity of patients with keratoconus in NZ might be greater than those in the USA in primary care, though this trend may also be observed at a tertiary level.

4.5 Conclusions

The results of the ARK Study: Part I suggest that keratoconus is relatively common in NZ with at least 1869 subjects in primary care over the 2-year study period. The majority of subjects are middle aged and approximately 90% have an established diagnosis of keratoconus suggesting a relatively low incidence. Most eyes had relatively poor UCVA; however, most attain acceptable BCVA using refractive correction. The average disease severity is skewed toward mild-moderate; however, 1 in 3 eyes used no correction, spectacles or stock soft contact lenses to attain BCVA. The results appear to support the long-held clinical impression that keratoconus is more prevalent in Māori and Pacific Peoples. Furthermore, it appears that Māori and Pacific patients may also suffer from more severe disease, and this may indicate a need for targeted screening of these populations in order to identify keratoconus earlier and offer appropriate interventions such as CXL.
Section 3:

The Aotearoa Research into Keratoconus (ARK) Study: Part II
The Natural History of Keratoconus in New Zealand

Chapters 5-8
Chapter 5:

The Natural History and Severity of Keratoconus in Participants with a Neophyte Diagnosis in the Auckland Region - The Aotearoa Research into Keratoconus (ARK) Study: Part IIA
5.1 Introduction

As discussed in chapter 2, the phenotypic characteristics of the keratoconic cornea have been investigated extensively over the last 160 years. However, there are few prospective longitudinal studies of the in vivo phenotypic changes that occur with this disease. Although the previously mentioned CLEK$^2$ and DUSKS$^3$ are landmark studies that have investigated how the characteristics of keratoconus changed in a longitudinal fashion, both studies were limited to only assessing corneal power/shape, and changes in refractive error and vision, largely due to limitations in technology available at their inception.

Historically, corneal power/shape and thickness are utilised to characterise the severity and progression of keratoconus, as the technology to measure these characteristics has existed since the 19th century (chapter 2). The earliest forms of these technologies, manual keratometry and optical pachymetry, have been utilised to produce grading scales for the severity of keratoconus such as the long-established Amsler-Krumeich scale.$^{229}$ The advent of corneal topography and corneal tomography has significantly advanced our understanding of the corneal power/shape and thickness features of keratoconus, however, the use of older grading scales such as the Amsler-Krumeich, which predates topo/tomography, is still utilised to classify disease severity in more recent tomography based studies of keratoconus.$^{235, 236}$

Current evidence suggests that corneal collagen cross-linking (CXL) has a greater likelihood for success when carried out in the mild-to-moderate stages of keratoconus.$^{237}$ Furthermore there is a widely-acknowledged, treatment safety limit of approximately 400µm minimum corneal thickness at the thinnest point,$^{238}$ thus early disease detection is essential. As a result, recent natural history studies of keratoconus focus on identifying when and who will progress from normal or keratoconus suspect to manifest keratoconus,$^{68, 224, 239}$ diagnosing keratoconus at an early stage,$^{240, 241}$ and characterising manifest keratoconus,$^{241-243}$ through the use of corneal topography and tomography.

Indeed, the wealth of research into the corneal tomographic characteristics of keratoconus has translated to clinical practice where assessment of progression and severity is generally focused on these aspects. However, newer technologies such as in vivo confocal microscopy (IVCM) and the CorVis ST (CST) now allow assessment of the corneal microstructure and biomechanical properties in a clinical setting. As noted in chapter 2, it has been established that a reduction in density of the sub-basal nerves and keratocytes is apparent in keratoconus.$^{95, 192}$ Similarly, the CST has revealed a detectable difference in biomechanical integrity of the cornea between normal eyes and those with keratoconus, though there is large overlap between the two groups.$^{244, 245}$ Despite the differences in
the corneal microstructure and biomechanical properties being relatively well characterised, it is unclear exactly what relationship they have to corneal tomographic measures of disease severity.

The ARK study: Part I (chapter 2) highlighted the higher transplantation rate for keratoconus in NZ, suggesting that it is either more common, more severe or progresses at a greater rate in the NZ population when compared internationally. While the ARK Study: Part I shed some light on the epidemiology and basic clinical characteristics of keratoconus in NZ, it had limitations in assessing disease severity and did not assess rates of progression. Indeed, very few studies have been conducted to assess the severity of keratoconus in NZ on a large scale, and none have assessed potential differences in progression rates among different ethnicities.

**Aims**

The aims of this study were to:

1) Characterise the corneal phenotypic features of keratoconus at initial diagnosis
2) Characterise the corneal phenotypic changes that occur as keratoconus progresses using advanced anterior segment imaging techniques
3) Compare disease characteristics/severity and rates of progression of keratoconus between ethnicities in New Zealand
4) Determine if changes in computerised corneal tomography occur prior to, concurrently with, or following changes in refractive error and vision, corneal microstructure and biomechanics

**5.2 Methods**

**5.2.1 Study Protocol and Design**

This study was designed as a prospective, longitudinal, observational investigation. Participants with a new diagnosis of keratoconus were examined at baseline, 8 months and 16 months. Each examination included assessment of: uncorrected vision, refractive error, best spectacle corrected visual acuity, corneal power and thickness (Pentacam HR), corneal microstructure (HRT II) and corneal biomechanics (CorVis ST). All outcome measures stated below were obtained as per the methodology outlined in Chapter 3, which includes details on how the IVCM images were captured and analysed. All research procedures were carried out within the University of Auckland clinical research laboratory located within the Ophthalmology Department at Greenlane Clinical Centre, ADHB, Auckland, New Zealand. Recruitment began in January 2014.
5.2.2 Outcome Measures

Computerised Corneal Tomography
- Steep simulated keratometry ($K_{\text{steep}}$) (D)
- Flat simulated keratometry ($K_{\text{flat}}$) (D)
- Maximum Corneal Power ($K_{\text{max}}$) (D)
- Central corneal thickness (CCT) (µm)
- Thinnest corneal thickness (TCT) (µm)

Refractive Error and Vision
- Manifest sphere (D)
- Manifest cylinder (D)
- Spherical equivalent (D)
- Uncorrected vision (UCVA) (LogMAR)
- Best spectacle corrected VA (BSCVA) (LogMAR)

In vivo Confocal Microscopy
- Corneal sub-basal nerve density (mm/mm²)
- Anterior keratocyte density (cells/mm²)
- Posterior keratocyte density (cells/mm²)

Corneal Biomechanics
- Deformation amplitude (DA) (mm)

5.2.3 Participants

Participants were recruited from within the Auckland region, both from optometrists in the wider community, and cornea and anterior segment sub-specialists at the Ophthalmology service within GLCC, ADHB. Regular recruitment announcements were sent out to optometrists via the newsletters distributed by the NZAO and CCLS (Appendix C). Similar to the ARK Study: Part I (chapter 4), this limb of the study was included in the author’s conference presentations on the ARK project at the NZAO and CCLS annual national conferences in 2013 and 2014 to further encourage recruitment.

This investigation adhered to the tenets of the Declaration of Helsinki and ethical approval to carry out the study was obtained from the University of Auckland Human Participant Ethics committee (UoAHPEC), approval number: 010549.
Inclusion Criteria
- Participants with a diagnosis of keratoconus <3 months prior to enrolment in the study were recruited. The diagnosis of keratoconus was confirmed at the baseline examination, based on the criteria specified in chapter 3
- The ability to understand the implications of the study, such that they were able to provide fully informed written consent or a parent/guardian was able to do so if the participant was less than 16 years of age

Exclusion Criteria
- Previous/current episode of corneal hydrops in either eye
- Previous ocular surgery or trauma
- Systemic disease which may affect the cornea (e.g. diabetes mellitus)
- Previous corneal herpetic disease
- Any other ocular pathology
- Inability to give informed consent
- Inability to attend follow-up appointments over the study period

Statistical Analysis
Each of the two eyes of each participant was classified as either “more severe” or “less severe” based on tomographic severity; the eye with higher $K_{\text{mean}}$ was designated the more severe eye, if $K_{\text{mean}}$ was the same, the eye with the lower TCT was designated the more severe eye.

The Kolmogorov-Smirnov test was performed to confirm that all study parameters were parametric. The independent samples t-test was used to analyse the difference in the outcome measures between the eyes of the participants. The Pearson correlation was used to determine if corneal tomographic parameters were correlated with refractive error and vision, corneal microstructure and biomechanics.

All analyses were performed using SPSS 22.0 for Windows (Chicago, IL, USA). A p-value of <0.05 was considered as significant throughout.
5.3 Results

Thirty eligible participants were recruited over a period of six months and all attended for baseline examination. Twenty eight (93.3%) participants were recruited through the anterior segment clinics at GLCC, and 2 (6.7%) participants were recruited through optometrists.

However, following their baseline visit, it was notable that 20 (66.7%) participants had undergone CXL in at least one eye. It also became rapidly apparent that the majority of subjects being referred were listed, or eligible, for CXL (which is provided by the GLCC, ADHB public service). It was therefore decided that it is no longer possible for the investigation to be completed 6-months following its initiation (June 2014) as the natural history was altered in over 50% of initial participants and it would be unethical to delay CXL treatment where indicated. Therefore, early in this limb of the ARK study recruitment and all follow-up reviews were ceased. Therefore, only baseline data are presented here.

Sixty eyes of 30 participants were included in the analyses; the mean age of the cohort was 20.8 ± 6.6 years, 15 (50%) male, 14 (46.7%) Pacific Peoples, 5 (16.7%) European, 4 (13.3%) Māori, 4 (13.3%) Asian and 3 (10.0%) Middle Eastern/Latin American/African.

5.3.1 Disease Severity

The results of the analyses of the severity of keratoconus in the more severe and less severe eyes of the 30 participants that underwent the baseline visit, contained in Table 5-1., suggests that keratoconus was asymmetric at diagnosis in the study participants. The more severe eye had a significantly higher mean corneal power (K\text{flat}, K\text{steep}, K\text{mean} and K\text{max}), lower mean corneal thickness (CCT and TCT), worse mean UCVA and BSCVA, lower mean sub-basal nerve, anterior and posterior keratocyte density, and a higher mean DA (p < 0.05 in all cases). There was no difference in the mean manifest sphere, manifest cylinder and spherical equivalent between the more severe and less severe eye.
Table 5-1 Descriptive statistics for main outcome measures for the more and less severe eye and difference between the two eyes (n = 60 eyes of 30 participants that underwent a baseline visit); flat simulated keratometry ($K_{\text{flat}}$), steep simulated keratometry ($K_{\text{steep}}$), mean simulated keratometry ($K_{\text{mean}}$), maximum corneal power ($K_{\text{max}}$), thinnest corneal thickness (TCT), central corneal thickness (CCT), unaided vision (UCVA), best spectacle corrected visual acuity (BSCVA), manifest sphere, manifest cylinder, spherical equivalent, sub-basal nerve density, anterior keratocyte density, posterior keratocyte density, deformation amplitude.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>More Severe Eye Mean ± std</th>
<th>Less Severe Eye Mean ± std</th>
<th>Difference Mean ± std</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{\text{flat}}$ (D)</td>
<td>48.4 ± 5.3</td>
<td>44.6 ± 4.0</td>
<td>3.8 ± 6.6</td>
<td>0.03†</td>
</tr>
<tr>
<td>$K_{\text{steep}}$ (D)</td>
<td>54.5 ± 7.3</td>
<td>48.3 ± 4.7</td>
<td>6.2 ± 8.5</td>
<td>&lt;0.01†</td>
</tr>
<tr>
<td>$K_{\text{mean}}$ (D)</td>
<td>51.4 ± 6.0</td>
<td>46.5 ± 4.2</td>
<td>4.9 ± 7.3</td>
<td>&lt;0.01†</td>
</tr>
<tr>
<td>$K_{\text{max}}$ (D)</td>
<td>61.7 ± 10.6</td>
<td>53.1 ± 7.2</td>
<td>8.6 ± 12.1</td>
<td>&lt;0.01†</td>
</tr>
<tr>
<td>CCT (µm)</td>
<td>462 ± 49</td>
<td>495 ± 48</td>
<td>33 ± 51</td>
<td>0.01†</td>
</tr>
<tr>
<td>TCT (µm)</td>
<td>434 ± 56</td>
<td>473 ± 48</td>
<td>39 ± 62</td>
<td>&lt;0.01†</td>
</tr>
<tr>
<td>UCVA (LogMAR)</td>
<td>1.0 ± 0.4</td>
<td>0.6 ± 0.4</td>
<td>0.4 ± 0.6</td>
<td>&lt;0.01†</td>
</tr>
<tr>
<td>BSCVA (LogMAR)</td>
<td>0.4 ± 0.3</td>
<td>0.2 ± 0.2</td>
<td>0.2 ± 0.3</td>
<td>&lt;0.01†</td>
</tr>
<tr>
<td>Manifest Sphere (D)</td>
<td>-3.10 ± 5.59</td>
<td>-1.23 ± 3.79</td>
<td>-1.87 ± 4.2</td>
<td>0.13</td>
</tr>
<tr>
<td>Manifest Cylinder (D)</td>
<td>-4.60 ± 2.26</td>
<td>-3.54 ± 2.32</td>
<td>-1.06 ± 2.8</td>
<td>0.08</td>
</tr>
<tr>
<td>Spherical Equivalent (D)</td>
<td>-5.40 ± 5.80</td>
<td>-3.00 ± 4.22</td>
<td>-2.40 ± 4.9</td>
<td>0.07</td>
</tr>
<tr>
<td>Sub-basal Nerve Density (mm/mm²)</td>
<td>15.4 ± 4.1</td>
<td>18.4 ± 4.4</td>
<td>-3.0 ± 3.6</td>
<td>&lt;0.01†</td>
</tr>
<tr>
<td>Anterior Keratocyte Density (Cells/mm²)</td>
<td>494 ± 127</td>
<td>585 ± 99</td>
<td>-91 ± 123</td>
<td>&lt;0.01†</td>
</tr>
<tr>
<td>Posterior Keratocyte Density (Cells/mm²)</td>
<td>267 ± 39</td>
<td>300 ± 34</td>
<td>-33 ± 49</td>
<td>&lt;0.01†</td>
</tr>
<tr>
<td>Deformation Amplitude (mm)</td>
<td>1.30 ± 0.15</td>
<td>1.13 ± 0.14</td>
<td>0.17 ± 0.19</td>
<td>&lt;0.01†</td>
</tr>
</tbody>
</table>

† Significant result
5.3.2 Correlation between Tomographic Disease Severity and Other Disease Characteristics

Table 5-2 highlights the relationship between the corneal tomographic parameters and refractive error, vision, microstructural and biomechanical parameters.

Corneal power ($K_{flat}$, $K_{steep}$, $K_{mean}$ and $K_{max}$) was significantly correlated with all other study parameters except manifest sphere. There were moderate-strong positive correlations with UCVA, BSCVA and DA ($r > 0.54$, $p < 0.01$, in all cases), while there were weak to strong negative correlations with manifest cylinder, spherical equivalent, sub-basal nerve density, anterior and posterior keratocyte density ($r = -0.238$ to $-0.699$, $p < 0.03$, in all cases).

Corneal thickness (CCT and TCT) was significantly correlated with all other study parameters. There were moderate-strong positive correlations with manifest sphere, manifest cylinder, spherical equivalent, sub-basal nerve density, anterior and posterior keratocyte density ($r > 0.39$, $p < 0.05$, in all cases), and strong negative correlations with UCVA, BSCVA and DA ($r < -0.57$, $p < 0.01$, in all cases).
Table 5-2 Pearson’s correlation between corneal tomographic parameters; flat simulated keratometry ($K_{\text{flat}}$), steep simulated keratometry ($K_{\text{steep}}$), mean simulated keratometry ($K_{\text{mean}}$), maximum cornea power ($K_{\text{max}}$), thinnest corneal thickness (TCT), central corneal thickness (CCT), and other disease characteristics; unaided vision (UCVA), best spectacle corrected visual acuity (BSCVA), manifest sphere, manifest cylinder, spherical equivalent, sub-basal nerve density, anterior keratocyte density, posterior keratocyte density, deformation amplitude. (n = 60 eyes of 30 participants that underwent a baseline visit)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter</th>
<th>Pearson’s correlation</th>
<th>$K_{\text{flat}}$ (D)</th>
<th>$K_{\text{steep}}$ (D)</th>
<th>$K_{\text{mean}}$ (D)</th>
<th>$K_{\text{max}}$ (D)</th>
<th>CCT (µm)</th>
<th>TCT (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCVA (LogMAR)</td>
<td>Pearson’s correlation</td>
<td>0.616</td>
<td>0.632</td>
<td>0.649</td>
<td>0.549</td>
<td>-0.628</td>
<td>-0.653</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>BSCVA (LogMAR)</td>
<td>Pearson’s correlation</td>
<td>0.592</td>
<td>0.788</td>
<td>0.751</td>
<td>-0.577</td>
<td>-0.633</td>
<td>P&lt;0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manifest Sphere (D)</td>
<td>Pearson’s correlation</td>
<td>-0.307</td>
<td>-0.331</td>
<td>-0.338</td>
<td>-0.238</td>
<td>0.409</td>
<td>0.392</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>P=0.02</td>
<td>P=0.01</td>
<td>P=0.01</td>
<td>P=0.07</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Manifest Cylinder (D)</td>
<td>Pearson’s correlation</td>
<td>-0.469</td>
<td>-0.589</td>
<td>-0.568</td>
<td>-0.572</td>
<td>0.482</td>
<td>0.509</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Spherical Equivalent (D)</td>
<td>Pearson’s correlation</td>
<td>-0.392</td>
<td>-0.442</td>
<td>-0.443</td>
<td>-0.351</td>
<td>0.490</td>
<td>0.481</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Sub-basal Nerve Density (mm/mm$^2$)</td>
<td>Pearson’s correlation</td>
<td>-0.376</td>
<td>-0.562</td>
<td>-0.502</td>
<td>-0.532</td>
<td>0.574</td>
<td>0.615</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Anterior Keratocyte Density (Cells/mm$^2$)</td>
<td>Pearson’s correlation</td>
<td>-0.390</td>
<td>-0.486</td>
<td>-0.462</td>
<td>-0.476</td>
<td>0.371</td>
<td>0.432</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P=0.04</td>
<td>P&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Posterior Keratocyte Density (Cells/mm$^2$)</td>
<td>Pearson’s correlation</td>
<td>-0.535</td>
<td>-0.699</td>
<td>-0.654</td>
<td>-0.686</td>
<td>0.567</td>
<td>0.585</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Deformation Amplitude (mm)</td>
<td>Pearson’s correlation</td>
<td>0.721</td>
<td>0.826</td>
<td>0.812</td>
<td>0.796</td>
<td>-0.604</td>
<td>-0.727</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>
5.4 Discussion

Anterior segment imaging technology has progressed significantly over the past 20 years, allowing for the phenotypic characteristics of keratoconus to be analysed in greater detail, creating greater potential to investigate methods of assessing disease severity and progression. As discussed in chapter 2, there has indeed been significant investigation into the characteristics of keratoconus utilising these imagining technologies, yet no studies have utilised several imaging technologies to determine how features such as corneal microstructure, biomechanics, power/shape and thickness might relate to each other and change as the disease progresses.

An intervention aimed at halting or slowing down the natural history of keratoconus (CXL) has recently become standard of care in New Zealand. As a result, the majority of participants in this study could not be followed up longer-term due to undergoing CXL after the baseline visit (some of these participants were included in the investigation of CXL in chapter 9). Thus the main aim of characterising the phenotypic features of keratoconus and how these features change as the disease progresses could not be achieved. Additionally, since the study was curtailed early in the recruitment stage, with no longitudinal follow-up data obtained, limited inferences can be drawn from data relating differences in disease severity or rate of progression between ethnicities, and comparisons with international studies.

In the context of the noted limitations, the results of this study suggest some interesting trends. On average, there is significant inter-eye asymmetry in the severity of keratoconus in the participant’s at diagnosis, with the worse eye having; greater corneal power/curvature and DA, lower CCT, TCT, sub-basal nerve density, anterior and posterior keratocyte density and poorer UCVA and BSCVA. The degree of inter-eye asymmetry is greater in this study than previous investigations that used the Pentacam: $K_{\text{flat}}; 3.8 \pm 6.6$ D vs. $2.7 \pm 3.3$ D, $K_{\text{steep}}; 6.2 \pm 8.5$ D vs. $3.8 \pm 4.2$ D, and $4.4 \pm 5.1$ D, CCT; $33 \pm 51$ µm vs. $26 \pm 24$ µm and $30 \pm 36$ µm. There may be a bias toward greater disease severity as this study was carried out at the same tertiary centre as the investigation of the tomographic characteristics of keratoconus by Jordan et al. which suggested that the majority of patients had severe disease (also discussed in chapter 4). Finally, Pacific Peoples participants (46.7%) made up the majority of our study group, Jordan et al. also showed disproportionate numbers of Pacific Peoples patients with keratoconus attending the corneal service at GLCC, which may further bias the disease severity – the ARK Study: Part I (chapter 4) demonstrated greater disease severity in Pacific Peoples participants.

There were no longitudinal follow-up data in this study due to the follow-up visits being curtailed for reasons previously outlined, however, relationships at diagnosis, and potentially the relationship to...
disease severity and progression, was investigated. The results indicated that corneal power/shape and thickness are significantly correlated with UCVA, BSCVA, refractive error, sub-basal nerve density, anterior and posterior keratocyte density and DA. Hypothetically, since all of the aforementioned parameters are likely/know to change with disease progression, these parameters may have potential to be utilised in conjunction with the “traditional” parameters to monitor disease progression. However, it is still unclear whether changes in corneal power/shape and thickness occur simultaneously, prior to or following changes in refractive error, vision, corneal microstructure and biomechanics, limiting the conclusions that can be drawn concerning their use in monitoring the progression of keratoconus.

The planned study was unable to be completed due to CXL becoming the standard of care for any participants that have evidence of progressive keratoconus. Unfortunately, it has become clear during the course of the inter-related studies that constitute this PhD thesis that the era of major prospective studies of the natural history of keratoconus, such as CLEK\(^2\) and DUSKS,\(^3\) may be over and future investigations of the natural history may be largely limited to retrospective studies.

### 5.5 Conclusions

This study suggests that keratoconus is typically asymmetric in corneal presentation, possibly more so in NZ than other parts of the world. Correlation between corneal power/shape and thickness and refractive error, vision, microstructure and biomechanics suggest that several parameters utilised in combination may provide a more comprehensive means of assessing progression. However, the inability to obtain longitudinal follow-up data limits the applicability of the findings.

Investigations of the natural history of keratoconus utilising the most up to date technological advances are still necessary and are extremely valuable in further developing the risk profile of progressive keratoconus. However, with CXL becoming standard of care in patients with progressive disease, prospective studies of the natural history of keratoconus are becoming increasingly difficult to conduct and may limit future investigations to retrospective analyses.
Chapter 6:

Assessment of the Natural History of Keratoconus through Retrospective, Longitudinal Analysis of Contact Lens Clinical Records
- The Aotearoa Research into Keratoconus (ARK) Study: Part IIB
6.1 Introduction

As discussed in chapter 5, prospective longitudinal studies of the natural history of keratoconus have become increasingly difficult to conduct since corneal collagen cross-linking became the standard of care in the treatment of progressive keratoconus. Retrospective analysis may therefore be the only ethically acceptable way of currently assessing the natural history of keratoconus in NZ.

In NZ, once the diagnosis of keratoconus has been made, typically patients usually remain under the care of primary care optometrists in the long term for refractive correction with either spectacles or contact lenses (CLs). Therefore, many patients with keratoconus have been followed-up for many years in the optometry setting, making it an ideal target area for retrospective analysis.

A limitation in the primary care optometry setting in NZ is that (expensive) technological advances may take significant time to permeate into practices; indeed a recent survey by the New Zealand Association of Optometrists (NZAO) revealed that just 31.6% of optometry practices had access to a computerised corneal topographer while 90.6% had a keratometer. However, corneal topography and keratometry are not the only means of quantifying corneal curvature. Along with keratometry, the CLEK study utilised a specialised set of rigid gas permeable (RGP) CLs to determine the lens that provided the first definite apical corneal clearance, termed the first definite apical clearance lens (FDACL), which was shown to be highly repeatable and comparable to keratometry.

RGP CLs as are usually manufactured based on proprietary design formulas but generally the greatest curvature or sagittal height of the lens is located in the optic zone diameter, termed the contact lens base curve (CLBC), with progressive flattening in curvature from the CLBC to the lens edge. In practice, several specialty RGP CL designs exist such as the Rose K and Boston Equalens variety of CLs, with each design employing different fitting principles, the three most common are apical bearing, three-point touch and apical clearance.

With the apical bearing CL/cornea relationship, the majority of the contact lens is supported by the corneal apex, a “flat-fitting” relationship between the cornea and CLBC; however, this fitting principle is rarely used in the modern era as it has been shown to traumatise the cornea, hastening apical scarring of the stroma, though contact lens wear in general has been shown to be predictive of corneal scarring. The three-point touch relationship is observed when there is minimal apparent contact between the corneal apex and CL, with the majority of the contact lens landing on the peripheral cornea spreading the load of the CL and minimising apical trauma. The three-point touch fitting relationship has been found to be the most popular amongst practitioners. Finally, the apical clearance relationship demonstrates total vaulting of the corneal apex by the CL with no
contact between the CLBC and cornea, the CL bearing all of its weight on the peripheral cornea.\textsuperscript{144} This relationship is the “steepest-fitting” of the three and is similar to the principle used in the CLEK to determine the FDACL.\textsuperscript{2}

Regardless of the fitting principle it is apparent that corneal curvature/power and CLBC are inextricably linked and therefore an increase in CLBC would be indicative of an increase in corneal curvature/power, measured with corneal topography or keratometry. Thus retrospective longitudinal assessment of change in corneal curvature/power may be measured using changes in a) keratometry b) computerised topography or c) CLBC as a surrogate measure. How such alterations in corneal curvature/power relate to changes in contact lens corrected visual acuity has significant potential to add to our knowledge of the natural history of keratoconus, particularly in NZ where there is a paucity of studies in this area. Fortunately, a contact lens subsidy is available to subjects with keratoconus who require CLs in NZ, provided by the New Zealand Ministry of Health (NZMOH). This may increase the likelihood of subjects with keratoconus in NZ utilising CLs long term as the cost of this treatment is lessened, thus providing useful very long term follow-up data.

**Aims**

The aims of this study were to:

1) Investigate longitudinal changes in RGP contact lens base curve and keratometry in subjects with keratoconus

2) Investigate the natural history of RGP contact lens corrected visual acuity in subjects with keratoconus

3) Determine if disease severity, in terms of RGP contact lens base curve and keratometry in subjects with keratoconus, is predictive of the visual acuity achieved with the contact lens corrected visual acuity of a given RGP contact lens
6.2 Methods

6.2.1 Study Protocol and Design
This study was a retrospective, longitudinal investigation. The clinical records of participants with keratoconus who successfully wore rigid contact lenses and underwent consultation between 1985 and 2015 at two sub-specialty optometry practices with more than 40 years of specialised provision of eye care for keratoconus in Auckland, New Zealand (Mortimer Hirst Eyecare and Eyewear (MH) and Visique Frith and Laird (FL)) were reviewed. Longitudinal data regarding CL-related clinical characteristics were collected at one year intervals following the participant’s initial consultation. Keratometry data were collected as MH acquired a corneal topographer in 2002 and FL in 2016. Thus long term longitudinal keratometry data, obtained with the manual Bausch and Lomb keratometer (Bausch & Lomb Inc, Rochester, New York, USA) at both practices, were more readily available.

6.2.2 Outcome Measures
- Contact lens base curve (CLBC) (mm converted to D using the conversion formula: dioptres = 337.5/mm )
- Contact lens corrected visual acuity (CLVA) (LogMAR)
- Keratometry
  - Steep keratometry ($K_{steep}$) (D)
  - Flat keratometry ($K_{flat}$) (D)
  - Mean keratometry ($K_{mean}$) (D)

6.2.3 Participants
The electronic and hard copy databases of both MH and FL were analysed to identify potential participants. All subjects coded as having keratoconus by a practitioner using the practice’s own coding method, receiving the NZMOH contact lens subsidy or identified as wearing a specialty keratoconus RGP design, were identified and subject to the selection criteria described below.

Ethical approval to carry out this study was obtained from the University of Auckland Human Participant Ethics committee (UoAHPEC) as an amendment to the ethical approval obtained for the ARK Study: Part I, approval number: 010547.
Inclusion Criteria
- Manifest keratoconus. As stated in chapter 3, the diagnosis of keratoconus was made by a community optometrist, on the basis of examination utilising a combination of keratometry, slit-lamp biomicroscopy and refraction
- Successful use of a rigid contact lens in at least one eye for ≥5 years
- At least 5 years of follow-up data

Exclusion Criteria
- Previous/current episode of acute corneal hydrops in either eye
- Forme fruste keratoconus
- Rigid contact lens intolerance with less than 5 years of successful wear
- Previous ocular surgery (including keratoplasty and corneal collagen cross-linking (CXL)) or trauma
  o Keratoplasty at presentation was an exclusion criterion, however, patients that received a keratoplasty >5 years after presentation were included and data included up until the keratoplasty.
- Systemic disease which may affect the cornea (e.g. diabetes mellitus)
- Previous corneal herpetic disease
- Any other ocular pathology

Statistical Analysis
Each eye of participants that met the inclusion criteria was considered separately for all analyses.

The Pearson correlation was used to determine if CLBC, CLVA, age and keratometry were correlated.

The Kolmogorov-Smirnov test was performed to confirm that all study parameters were parametric. Participants were grouped by age and follow-up duration. The repeated measures ANOVA and Bonferroni post-hoc analysis was used to analyse differences in outcome measures between consecutive age groups. (<20, 20-30, 30-40, 40-50 and >60 years).

To assess CLVA as a function of disease severity based on corneal curvature/power, CLBC and K_{steep} were grouped empirically at 5D intervals, K_{flat} according to the “Gold Standard” grading scheme for keratoconus outlined in the CLEK study and K_{mean} according to the Amsler-Krumeich grading scale for keratoconus, each grading scheme only takes K_{flat} and K_{mean} into account, respectively. Any eye at any time point that had both CLVA and the parameter being assessed was used. The one-way ANOVA and Tukey post-hoc analysis were used to analyse differences in CLVA between the CLBC, K_{steep}, K_{flat} and K_{mean} groups.
CLBC, K\text{steep}, K_{\text{flat}} and K_{\text{mean}} were compared between eyes that obtained a CLVA \leq 0.3 LogMAR (better than 6/12) and >0.3 LogMAR (worse than 6/12) using the independent samples t-test. The mean CLBC, K_{\text{steep}}, K_{\text{flat}} and K_{\text{mean}} of eyes that obtained a CLVA \leq 0.3 LogMAR (better than 6/12) were utilised as categorical predictors of CLVA >0.3 LogMAR in a binomial logistic regression analysis to produce odds ratios (ORs) and 95% confidence intervals (CIs).

All analyses were performed using SPSS 22.0 for Windows (Chicago, IL, USA). A p-value of <0.05 was considered as significant throughout.

### 6.3 Results

The clinical records of 626 potential participants with keratoconus were identified, 406 of which were included in the final analyses. Of the 220 potential participants that were excluded, the majority either did not have at least 5 years of follow-up data (136 (61%)) or were intolerant to RGP CLs (51 (23%)), and the remainder had bilateral corneal transplantations at presentation (33 (15%)). The baseline characteristics of the study group are shown in Table 6-1. Data from 642 (79%) eyes of the 406 eligible participants were collected. Of the excluded eyes, 146 (18%) had a corneal transplant at presentation, 16 (2%) had a history of acute corneal hydrops and 8 (1%) had other ocular pathology.

Table 6-1 Characteristics of the study cohort at their initial presentation: age, gender, follow-up period, contact lens base curve (CLBC), steep keratometry (K_{\text{steep}}), flat keratometry (K_{\text{flat}}), mean keratometry (K_{\text{mean}}) and contact lens corrected visual acuity (CLVA). N = 642 eyes of 406 participants

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Mean ± SD or N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31.9 ± 12.9</td>
</tr>
<tr>
<td>Male</td>
<td>227 (56%)</td>
</tr>
<tr>
<td>Female</td>
<td>179 (44%)</td>
</tr>
<tr>
<td>Follow-up Period (years)</td>
<td>10.2 ± 4.8 years (range 5-30)</td>
</tr>
<tr>
<td>CLBC (D)</td>
<td>48.9 ± 4.7</td>
</tr>
<tr>
<td>K_{\text{steep}} (D)</td>
<td>49.6 ± 5.1</td>
</tr>
<tr>
<td>K_{\text{flat}} (D)</td>
<td>45.6 ± 3.8</td>
</tr>
<tr>
<td>K_{\text{mean}} (D)</td>
<td>47.6 ± 4.2</td>
</tr>
<tr>
<td>CLVA (LogMAR)</td>
<td>0.17 ± 0.21</td>
</tr>
</tbody>
</table>
6.3.1 Pearson Correlations
Table 6-2 contains the results of the Pearson’s correlations between CLBC, CLVA, age and keratometry. CLBC had a significant strong positive correlation with $K_{\text{Steep}}$, $K_{\text{flat}}$, and $K_{\text{mean}}$ ($r > 0.8$ and $p < 0.001$ in all cases) and a significant but weak-moderate positive correlation with CLVA ($r = 0.379$, $p < 0.001$). CLBC, $K_{\text{Steep}}$, $K_{\text{flat}}$, and $K_{\text{mean}}$ all had significant but weak positive correlations with age while $K_{\text{Steep}}$, $K_{\text{flat}}$, and $K_{\text{mean}}$ had a significant but weak-moderate positive correlation with CLVA.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>Age (years)</th>
<th>N</th>
<th>CLVA (LogMAR)</th>
<th>N</th>
<th>CLBC (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLBC (D)</td>
<td>1158</td>
<td>$r=0.113$</td>
<td>2017</td>
<td>$r=0.379$</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>$K_{\text{steep}}$ (D)</td>
<td>1784</td>
<td>$r=0.079$</td>
<td>831</td>
<td>$r=0.070$</td>
<td>1171</td>
<td>$r=0.803$</td>
</tr>
<tr>
<td>$K_{\text{flat}}$ (D)</td>
<td></td>
<td>$p&lt;0.001$</td>
<td></td>
<td>$p=0.044$</td>
<td></td>
<td>$p&lt;0.001$</td>
</tr>
<tr>
<td>$K_{\text{mean}}$ (D)</td>
<td></td>
<td>$r=0.195$</td>
<td></td>
<td>$r=0.073$</td>
<td></td>
<td>$r=0.850$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$p&lt;0.001$</td>
<td></td>
<td>$p=0.035$</td>
<td></td>
<td>$p&lt;0.001$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$r=0.136$</td>
<td></td>
<td>$r=0.074$</td>
<td></td>
<td>$r=0.857$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$p&lt;0.001$</td>
<td></td>
<td>$p=0.033$</td>
<td></td>
<td>$p&lt;0.001$</td>
</tr>
</tbody>
</table>
6.3.2 Change Over Time

Table 6-3 and Figures 6-1 and 6-2; demonstrate the change in the main outcome measures over time. The mean CLBC increased significantly between all age groups <50 years but did not change significantly between age groups >50 years. CLVA only reduced significantly between age groups 40 – 50 and 50 – 60 years. $K_{\text{steep}}$, $K_{\text{flat}}$ and $K_{\text{mean}}$ only reduced significantly between age groups <20 and 20 – 30 years.

Table 6-3 Change in contact lens base curve (CLBC), steep keratometry ($K_{\text{steep}}$), flat keratometry ($K_{\text{flat}}$) and mean keratometry ($K_{\text{mean}}$) between age groups. The p-values represent the result of the repeated measures ANOVA and Bonferroni post-hoc analyses between age groups. Only participants that were present in each of the two age groups being assessed were included in the analysis thus each age group appears twice except for <20 and >60 years. All parameter values are mean ± standard deviation.

<table>
<thead>
<tr>
<th>Age Group (years)</th>
<th>CLBC (D)</th>
<th>CLVA (LogMAR)</th>
<th>$K_{\text{steep}}$ (D)</th>
<th>$K_{\text{flat}}$ (D)</th>
<th>$K_{\text{mean}}$ (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>48.32 ± 4.08</td>
<td>0.18 ± 0.23</td>
<td>48.92 ± 4.44</td>
<td>45.50 ± 2.15</td>
<td>47.21 ± 3.12</td>
</tr>
<tr>
<td>20 – 30</td>
<td>49.91 ± 4.01</td>
<td>0.19 ± 0.09</td>
<td>53.23 ± 6.08</td>
<td>48.15 ± 3.56</td>
<td>50.69 ± 4.66</td>
</tr>
<tr>
<td></td>
<td>p=0.001</td>
<td>p=0.887</td>
<td>p=0.003</td>
<td>p=0.001</td>
<td>p=0.002</td>
</tr>
<tr>
<td></td>
<td>N=24</td>
<td>N=11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 – 30</td>
<td>47.86 ± 3.5</td>
<td>0.11 ± 0.11</td>
<td>50.42 ± 5.61</td>
<td>46.15 ± 4.09</td>
<td>48.28 ± 4.70</td>
</tr>
<tr>
<td>30 – 40</td>
<td>49.18 ± 3.7</td>
<td>0.08 ± 0.11</td>
<td>49.79 ± 5.25</td>
<td>46.53 ± 4.17</td>
<td>48.16 ± 4.56</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p=0.412</td>
<td>p=0.194</td>
<td>p=0.413</td>
<td>p=0.772</td>
</tr>
<tr>
<td></td>
<td>N=51</td>
<td>N=13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 – 40</td>
<td>48.95 ± 4.36</td>
<td>0.14 ± 0.12</td>
<td>49.04 ± 3.72</td>
<td>46.02 ± 3.05</td>
<td>47.53 ± 3.17</td>
</tr>
<tr>
<td>40 – 50</td>
<td>49.57 ± 4.67</td>
<td>0.14 ± 0.15</td>
<td>49.60 ± 3.70</td>
<td>46.66 ± 3.17</td>
<td>48.13 ± 3.28</td>
</tr>
<tr>
<td></td>
<td>p=0.017</td>
<td>p=0.933</td>
<td>p=0.142</td>
<td>p=0.103</td>
<td>p=0.090</td>
</tr>
<tr>
<td></td>
<td>N=60</td>
<td>N=32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 – 50</td>
<td>48.71 ± 3.74</td>
<td>0.12 ± 0.15</td>
<td>50.33 ± 2.7</td>
<td>47.00 ± 1.94</td>
<td>48.67 ± 2.02</td>
</tr>
<tr>
<td>50 – 60</td>
<td>49.07 ± 4.47</td>
<td>0.19 ± 0.13</td>
<td>50.20 ± 2.30</td>
<td>47.75 ± 2.40</td>
<td>48.97 ± 2.23</td>
</tr>
<tr>
<td></td>
<td>p=0.331</td>
<td>p=0.044</td>
<td>p=0.704</td>
<td>p=0.135</td>
<td>p=0.250</td>
</tr>
<tr>
<td></td>
<td>N=30</td>
<td>N=22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 – 60</td>
<td>50.70 ± 4.7</td>
<td>0.24 ± 0.11</td>
<td>51.95 ± 6.26</td>
<td>49.14 ± 5.48</td>
<td>50.54 ± 5.85</td>
</tr>
<tr>
<td>&gt;60</td>
<td>51.03 ± 4.8</td>
<td>0.24 ± 0.11</td>
<td>53.65 ± 9.70</td>
<td>49.80 ± 6.98</td>
<td>51.72 ± 8.33</td>
</tr>
<tr>
<td></td>
<td>p=0.400</td>
<td>p=0.936</td>
<td>p=0.341</td>
<td>p=0.489</td>
<td>p=0.372</td>
</tr>
<tr>
<td></td>
<td>N=20</td>
<td>N=8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Page | 109
Figure 6-1 Comparison of the mean contact lens base curve (CLBC) and contact lens corrected visual acuity (CLVA) between age groups A. Contact lens base curve B. Contact lens corrected visual acuity
Figure 6-2 Comparison of the mean steep keratometry (K_{steep}), flat keratometry (K_{flat}) and mean keratometry (K_{mean}) between age groups A. Steep keratometry B. Flat keratometry C. Mean keratometry
6.3.3 Contact Lens Corrected Visual Acuity with Increasing Corneal Curvature/Power

The mean CLVA decreased significantly in eyes with a CLBC of 48.0 – 53.0D compared to eyes with a CLBC of 43.0 – 48.0D and <43.0D, and continued to reduce significantly at CLBC 53.0 – 58.0D and >58.0D compared to all other groups. The mean CLVA for $K_{\text{steep}}$, $K_{\text{flat}}$ and $K_{\text{mean}}$ decreased with increasing corneal curvature/power and there was a significant difference between all groups except grades II and III in all cases (Table 6-4).

Table 6-4 Comparison of mean contact lens corrected visual acuity (CLVA) with increasing corneal curvature/power indicated by groups of incremental increase in curvature/power in contact lens base curve (CLBC), steep keratometry ($K_{\text{steep}}$), flat keratometry ($K_{\text{flat}}$) and mean keratometry ($K_{\text{mean}}$). All parameter values are mean ± standard deviation.

<table>
<thead>
<tr>
<th>Contact Lens Corrected Visual Acuity with Increasing Corneal Curvature/power</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLBC (D (mm))</td>
<td>&lt;43.0 (7.8)</td>
<td>43.0 – 48.0 (7.8 – 7.0)</td>
<td>48.0 – 53.0 (7.0 – 6.4)</td>
<td>53.0 – 58.0 (6.4 – 5.8)</td>
<td>&gt;58.0 (5.8)</td>
</tr>
<tr>
<td>CLVA (LogMAR)</td>
<td>0.15 ± 0.17 p=0.799</td>
<td>0.13 ± 0.12 p=0.783</td>
<td>0.17 ± 0.14 II-III p&lt;0.001 II-IV p&lt;0.001 I-III p&lt;0.001</td>
<td>0.23 ± 0.16 III-IV p&lt;0.001 II-IV p&lt;0.001 I-IV p&lt;0.001</td>
<td>0.39 ± 0.19 IV-V p&lt;0.001 III-V p&lt;0.001 II-V p&lt;0.001 I-V p&lt;0.001</td>
</tr>
<tr>
<td>N</td>
<td>102</td>
<td>823</td>
<td>726</td>
<td>268</td>
<td>99</td>
</tr>
<tr>
<td>Ksteep (D)</td>
<td>&lt;48.0</td>
<td>48.0 – 53.0</td>
<td>53.0 – 58.0</td>
<td>58.0 – 63.0</td>
<td>&gt;63.0</td>
</tr>
<tr>
<td>CLVA (LogMAR)</td>
<td>0.11 ± 0.12 l-II p=0.009</td>
<td>0.14 ± 0.12 l-II p=0.009</td>
<td>0.16 ± 0.11 II-III p=0.594 II-IV p&lt;0.001 I-III p&lt;0.001</td>
<td>0.20 ± 0.12 III-IV p=0.019 II-IV p&lt;0.001 I-IV p&lt;0.001</td>
<td>0.29 ± 0.20 IV-V p&lt;0.034 III-V p&lt;0.003 II-V p&lt;0.003 I-V p&lt;0.001</td>
</tr>
<tr>
<td>N</td>
<td>273</td>
<td>325</td>
<td>143</td>
<td>56</td>
<td>34</td>
</tr>
<tr>
<td>“Gold Standard” Grade</td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>IV</td>
<td></td>
</tr>
<tr>
<td>Kflat (D)</td>
<td>&lt;48.0</td>
<td>48.0 – 51.0</td>
<td>51.0 – 56.0</td>
<td>&gt;56.0</td>
<td></td>
</tr>
<tr>
<td>CLVA (LogMAR)</td>
<td>0.12 ± 0.12 l-II p=0.002</td>
<td>0.15 ± 0.12 l-II p=0.002</td>
<td>0.18 ± 0.13 II-III p=0.355 I-III p&lt;0.001</td>
<td>0.30 ± 0.18 III-IV p&lt;0.001 II-IV p&lt;0.001 I-IV p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>486</td>
<td>213</td>
<td>94</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Amsler-Krumeich Grade</td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>IV</td>
<td></td>
</tr>
<tr>
<td>Kmean (D)</td>
<td>&lt;48.0</td>
<td>48.0 – 53.0</td>
<td>53.0 – 55.0</td>
<td>&gt;55.0</td>
<td></td>
</tr>
<tr>
<td>CLVA (LogMAR)</td>
<td>0.11 ± 0.12 l-II p&lt;0.001</td>
<td>0.15 ± 0.13 l-II p&lt;0.001</td>
<td>0.16 ± 0.13 II-III p=0.951 I-III p&lt;0.001</td>
<td>0.24 ± 0.16 III-IV p&lt;0.001 II-IV p&lt;0.001 I-IV p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>356</td>
<td>340</td>
<td>42</td>
<td>93</td>
<td></td>
</tr>
</tbody>
</table>
6.3.4 Reduction in Contact lens corrected visual acuity below 6/12
Table 6-5 demonstrates the difference in the mean CLBC, K\text{StEEP}, K\text{fLAT} and K\text{MEEAN} between eyes with a CLVA ≤0.3 LogMAR (better than 6/12) and >0.3 LogMAR (worse than 6/12). The mean corneal curvature/power was significantly greater in eyes with a CLVA >0.3 LogMAR as indicated by a greater mean CLBC, K\text{StEEP}, K\text{fLAT} and K\text{MEEAN}, compared to eyes with a CLVA ≤0.3 LogMAR. CLBC, K\text{StEEP}, K\text{fLAT} and K\text{MEEAN} were all significant predictors of a CLVA >0.3 LogMAR, however, CLBC had the highest odds ratio (Table 6-6).

Table 6-5 Comparison of mean contact lens base curve (CLBC), steep keratometry (K\text{StEEP}), flat keratometry (K\text{fLAT}) and mean keratometry (K\text{MEEAN}) between eyes with a contact lens corrected visual acuity (CLVA) ≤0.3 and >0.3 LogMAR. All parameter values are mean ± standard deviation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>CLVA (LogMAR) ≤0.3 (≤6/12)</th>
<th>N</th>
<th>CLVA (LogMAR) &gt;0.3 (&gt;6/12)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLBC (D)</td>
<td>1805</td>
<td>48.20 ± 4.16</td>
<td>48.20 ± 4.16</td>
<td>213</td>
<td>52.92 ± 6.12</td>
</tr>
<tr>
<td>K\text{StEEP}(D)</td>
<td>768</td>
<td>50.83 ± 5.50</td>
<td>50.83 ± 5.50</td>
<td>64</td>
<td>54.73 ± 7.95</td>
</tr>
<tr>
<td>K\text{fLAT} (D)</td>
<td>47.28 ± 4.43</td>
<td>47.28 ± 4.43</td>
<td>50.69 ± 7.26</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>K\text{MEEAN} (D)</td>
<td>49.06 ± 4.78</td>
<td>49.06 ± 4.78</td>
<td>52.71 ± 7.43</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Table 6-6 Contact lens base curve (CLBC), steep keratometry (K\text{StEEP}), flat keratometry (K\text{fLAT}) and mean keratometry (K\text{MEEAN}) as predictors of a contact lens corrected visual acuity (CLVA) >0.3 LogMAR

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Odds Ratio</th>
<th>95% CI for Odds Ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLBC &gt;48.0D (&lt;7.0mm)</td>
<td>3.66</td>
<td>2.64</td>
<td>5.07</td>
</tr>
<tr>
<td>K\text{StEEP}&gt;51.0D</td>
<td>2.34</td>
<td>1.38</td>
<td>3.98</td>
</tr>
<tr>
<td>K\text{fLAT}&gt;47.0D</td>
<td>1.93</td>
<td>1.14</td>
<td>3.28</td>
</tr>
<tr>
<td>K\text{MEEAN}&gt;49.0D</td>
<td>2.68</td>
<td>1.54</td>
<td>4.67</td>
</tr>
</tbody>
</table>

6.4 Discussion
As discussed in chapters 2 and 5, longitudinal characterisation of the natural history of keratoconus has been investigated for over 160 years, however, it has received surprisingly little attention in the modern era. Indeed, the landmark CLEK\textsuperscript{2} and DUSKS\textsuperscript{232} bear the most resemblance to the current study though the CLEK is the most comparable as, similar to this study, it employed keratometry and the contact lens fitting characteristics to assess corneal curvature/power longitudinally.\textsuperscript{253}
The current study utilised longitudinal assessment of changes in contact lens base curve as a surrogate measure of changes in corneal curvature/power, which suggests that corneal curvature/power continues to increase up until age 40 – 50 years in patients with keratoconus (Table 6-2). Indeed, the mean CLBC increased significantly between age group pairs until the 40 – 50 and 50 – 60 years and 50 – 60 and >60 years groups were compared. The changes noted in CLBC are in line with the CLEK study which noted that the proportion of patients that had a change of ≥3.00D in FDACL in at least one eye, reduced progressively from 56.7% in the <20 years old age group to 9.4% in the 50 – 60 years old age group. However, the keratometric parameters did not follow the same pattern as CLBC in the current study, with $K_{\text{steep}}$, $K_{\text{flat}}$ and $K_{\text{mean}}$ all only increasing significantly between the <20 and 20 – 30 years old age groups. This is an unexpected result as this study demonstrated that $K_{\text{steep}}$, $K_{\text{flat}}$ and $K_{\text{mean}}$ are strongly correlated with CLBC, while the CLEK study also showed $K_{\text{steep}}$ and $K_{\text{flat}}$ to be highly correlated with FDACL, suggesting that an increase in CLBC would be accompanied by a similar change in keratometric parameters. A potential reason for the lack of increase in keratometric parameters with age, and thus increasing disease severity, may be attributed to the repeatability of manual keratometry. Indeed, manual keratometry has been shown to be repeatable and comparable to Scheimpflug based computerised corneal topography in normal eyes and those with mild keratoconus, however, as the severity of keratoconus increases the repeatability of manual keratometry reduces to a greater extent than was observed in the Scheimpflug system. Furthermore, the increase in corneal curvature/power that occurs in keratoconus does not always occur in the central cornea, the area which keratometric measurements are limited to. Another possibility is that the contact lens wear may have caused corneal warpage, resulting in artificially low keratometric readings.

It is noteworthy that both this study and the CLEK study showed that corneal curvature/power continued to increase beyond age 30 years, which is in contrast to the traditional view of keratoconus stabilising in the fourth decade of life. The current study highlights that CLBC and the keratometric parameters were only weakly correlated with age despite demonstrating an increase with age, suggesting a non-linear relationship which may be the result of a plateau in corneal curvature/power and disease progression ceasing, or slowing, with increasing age. Indeed, the CLEK study reported that the rate of change in corneal curvature/power was non-linear, reducing with increasing age but never ceasing completely. The topic of progression in keratoconus beyond age 30 is covered specifically in chapter 7.

The current study showed that CLVA did not reduce until age 50 – 60 years old, despite a trend of increasing corneal curvature/power with age. However, assessment of CLVA in relation to corneal curvature/power suggests that the two are closely linked, as expected. The results of this study
suggest that CLVA does not reduce significantly until the CLBC increases to 48.0 – 53.0D in patients with keratoconus, after which CLVA continues to reduce. Interestingly this appears to correlate with \( K_{\text{Steep}}, K_{\text{flat}}, \) and \( K_{\text{mean}} \) as CLVA reduced significantly when all three parameters increased above 48.0D (Table 6-3). Curiously, CLVA continued to reduce as \( K_{\text{Steep}}, K_{\text{flat}}, \) and \( K_{\text{mean}} \) increased except between grades II and III in all cases, suggesting that the relationship is not linear and somewhat validating the “Gold Standard”\(^{252}\) and Amsler-Krumeich\(^{229}\) grading scales as CLVA reduced significantly between 3 of the 4 grades, indicative of increasing disease severity between the grades.

Further evidence for a non-linear relationship between CLVA and corneal curvature/power in keratoconus is provided by the Pearson correlations which found that CLBC, \( K_{\text{Steep}}, K_{\text{flat}}, \) and \( K_{\text{mean}} \) were only weakly correlated with CLVA despite a distinct change with increasing corneal curvature/power. Notably, in all analyses of CLVA in relation to curvature/power, the mean reduction in CLVA was predominantly less than 0.1 LogMAR (1 line), indicating that the reduction in visual acuity with increasing curvature/power occurs in small increments. Indeed, the CLEK study reported that the reduction in high-contrast best corrected visual acuity (BCVA) occurred at a rate of only 0.29 ± 1.5 letters per year with a gradual reduction over the 8-year study period.\(^{257}\)

Considering the slow incremental reduction in CLVA with corneal curvature/power, an analysis of corneal curvature/power in keratoconus when CLVA drops below a certain level is useful. In this study the authors chose a CLVA of 0.3 LogMAR (6/12) as this is the driving license standard in NZ. The results indicate that corneal curvature/power is significantly lower in eyes that achieve a CLVA of ≤0.3 LogMAR as indicated by a lower mean CLBC, \( K_{\text{Steep}}, K_{\text{flat}}, \) and \( K_{\text{mean}} \). The mean CLBC, \( K_{\text{Steep}}, K_{\text{flat}}, \) and \( K_{\text{mean}} \) of eyes achieving ≤0.3 LogMAR were used as categorical criteria for assessing the likelihood of producing a CLVA of >0.3 LogMAR as these values likely represent a level near the maximum corneal curvature/power at which a CLVA of ≤0.3 LogMAR can be achieved as there is significant overlap of the 95% CI’s for CLBC, \( K_{\text{Steep}}, K_{\text{flat}}, \) and \( K_{\text{mean}} \) between the groups with CLVA ≤0.3 LogMAR and >0.3 LogMAR. The result of these analyses suggest that increased corneal curvature/power is highly predictive of CLVA, demonstrating significantly increased odds of a reduction in CLVA to >0.3 LogMAR, with CLBC >48.0D (<7.0 mm) having the greatest increased odds (OR 3.66). The CLEK study reported a similar relationship between increasing corneal curvature/power and BCVA, with an increase in FDACL carrying an OR of 1.07/D of losing ≥10 letters of BCVA in at least one eye.\(^{257}\)

Although retrospective studies have limitations, they have some advantages with respect to follow-up data over prospective investigations such as the CLEK where very long term follow-up is prone to participant drop out. Indeed, in the current study some participants had significantly longer follow-up (up to 30 years), compared to the CLEK study (8 years). However, an inherent limitation of a
longitudinal, retrospective analysis is that participants are not on a set, regular timescale for return examinations. In this study all participants had at least 5 years of follow-up data, however, in many cases the time between consultations was ≥3 years and in some cases ≥5 years. Thus, rates of change in the study parameters could not be calculated meaningfully. Utilising data points more than 1-2 years apart are subject to the limitation that the keratoconus may have progressed between data points but stopped a significant length of time before the second data point. Analysis of rates of change in the study parameters would have been useful as these could be directly compared to those reported in the CLEK study.253 The current study cohort does however, appear to have lower disease severity compared to the CLEK study as indicated by the lower mean $K_{\text{flat}}$ at baseline (45.6 ± 3.8 D vs. 49.5 ± 6.0 D48) which may affect disease progression rates, though the CLEK only report $K_{\text{flat}}$ in the worse eye.

Limitations in the follow-up data also affected the longitudinal assessment of all study parameters as in order for the repeated measures ANOVA to be performed, participants were required to have follow-up that spanned both age groups being assessed, resulting in small sample sizes. The longitudinal analyses of CLBC had the largest sample sizes making differences easier to detect. The smaller sample sizes in the longitudinal analysis of keratometry and CLVA may have contributed to similar related patterns of increasing CLBC not being observed in the keratometric parameters and CLVA. A pattern of increasing corneal curvature/power and reduction in CLVA with age is one we would expect to see, especially as subsequent analyses suggest that changes in all the study parameters are related to one another.

Another limitation in this retrospective analysis is that the presence/influence of commonly known associations of keratoconus, (including ethnicity, as indicated by ARK Part I – chapter 4, family history, eye rubbing and atopy etc.), on longitudinal changes in corneal curvature/power could not be investigated due to variations in recorded history. Furthermore, corneal curvature/power is not the sole determinant of CLVA. Factors such as anterior corneal stromal scarring are known to affect BCVA258 but could not be investigated due to variations in clinical record documentation. There is also a limitation in all analyses of this study as they were carried out using all eligible eyes with available data; when data from only one eye is available due to the other eye being ineligible for analysis, the change in study parameters over time and their effect on CLVA in the other eye is unknown, potentially biasing the results toward lower or greater change/effect. However, averaging the data from both eyes would have the potential to create a similar bias as keratoconus is known to be asymmetric.259 thus one eye may have greater influence than the other on the average of the two. Thus the results of this study are more indicative of the patterns seen in individual eyes with keratoconus as opposed to individuals with keratoconus (also discussed in chapter 4).
Interpretations of the patterns of change in corneal curvature/power should be considered carefully. Earlier, the limitations of the reduced repeatability of manual keratometry with increasing severity of keratoconus and corneal warpage on obtaining accurate keratometry measurements have been mentioned. The CL fitting relationship to the cornea can be also be a misleading method of assessing disease progression as if the lens is fitted with excessive apical clearance then the keratoconus has to progress a greater amount before a change in CLBC is required, leading to the appearance that the disease is progressing at a lower rate. It is therefore evident that both CLBC and keratometry are not necessarily sensitive enough to detect subtle disease progression. Additionally both techniques are limited to only assessing anterior corneal curvature/power, while changes in corneal thickness\textsuperscript{260} and posterior elevation\textsuperscript{64} are detectable in early keratoconus. Thus computerised corneal tomographers are the best current methods for assessing the progression of keratoconus as these devices also assess corneal thickness and posterior corneal elevation, changes in which may occur prior to changes in the anterior cornea.\textsuperscript{65} While subject to the same limitations explored here, retrospective investigations characterising the tomographic changes that occur as keratoconus progresses may yet reveal much about the disease process. Indeed a recent longitudinal retrospective investigation utilising computerised corneal tomography revealed several previously undescribed tomographic parameters predictive of disease progression.\textsuperscript{261}

6.5 Conclusions

Despite the inherent limitations of retrospective studies, this study provides considerable insight into the natural history of keratoconus, particularly the longitudinal changes that occur in corneal curvature/power and how disease severity in terms of corneal curvature/power relates to CLVA. The results of this study suggest that corneal curvature/power continues to increase in those with keratoconus up until the age of 40 – 50 years as indicated by changes in CLBC. While CLVA only reduces significantly once the CLBC increases to 48.0 – 53.0D (reduces to 7.0 – 6.4mm) thereafter it continues to decrease incrementally as the CLBC increases. An increase in $K_{\text{Steep}}, K_{\text{flat}}$ and $K_{\text{mean}}$ above 48D is also suggestive of a similar reduction in CLVA. Furthermore, higher corneal curvature/power as indicated by CLBC, $K_{\text{Steep}}, K_{\text{flat}}$ and $K_{\text{mean}}$ appears to increase the odds of an associated CLVA less than 6/12, with a CLBC >48.0D (<7.0mm) carrying the greatest increased odds (OR 3.66).

The results of investigating the relationship between contact lens corrected visual acuity and corneal curvature/power, as assessed by CLBC and keratometry in particular, have valuable implications for clinical practice. These data can be used to produce an indication of the potential CLVA that may be attained with a given CLBC, keratometric values, or change in these parameters, providing an evidence base from which practitioner and patient expectations can be formed.
Chapter 7:

The Natural History of Corneal Topographic Progression of Keratoconus after Age 30 years in Non-contact Lens Wearers - The Aotearoa Research into Keratoconus (ARK) Study: Part IIC
7.1 Introduction

As explored in ARK Part IIB (chapter 6), the traditional view of the natural history of keratoconus is; onset in the early teenage years with progression up until the fourth or fifth decade when it generally arrests. However, as suggested by the results of ARK Part IIB and the Collaborative Longitudinal Evaluation of Keratoconus (CLEK), it is possible that keratoconus continues to progress beyond the fourth decade. Typically, as keratoconus progresses the corneal curvature/power increases and while both ARK Part IIB and the CLEK demonstrate an increase in corneal curvature beyond age 30, both investigations utilised keratometry and the fitting relationship of rigid gas permeable (RGP) contact lenses (CLs) to measure corneal curvature/power. As previously explored in chapters 2 and 6, corneal curvature was traditionally measured using manual keratometry, however, these devices utilise just four measurements localised to the para-central cornea and have largely been superseded by computerised corneal topography/tomography devices, which utilise several thousand data points over much of the corneal surface, providing a more detailed assessment. Furthermore, as also explored in ARK Part IIB, the prolonged use of RGP CLs, a requirement for participants in ARK Part IIB while 69.2% of eyes utilised RGP CLs in the CLEK, has the potential to distort the shape of the cornea (corneal warpage), affecting measurements of curvature/power made using either keratometry or topography.

In light of the limitations imposed by the use of manual keratometry and prolonged RGP CL use in ARK Part IIB and the CLEK, it is apparent that the full natural history of keratoconus is yet to be determined definitively, particularly beyond age 30. As indicated in chapter 2 and ARK Part I (chapter 4) keratoconus is a relatively common disease affecting 8.6–2340/100,000 of the population worldwide. Furthermore, the mean age of the cohort in ARK Part I and CLEK was greater than 30 years at 41.0 ± 15.7 and 39.3 ± 10.9 years, respectively, which suggests that there a considerable number of middle aged and older subjects with keratoconus currently managed by optometrists in NZ and around the world. Hence, better knowledge of the risk of significant disease progression in this age-group would be beneficial for both those affected and health care providers, in terms of determining the prognosis and planning follow-up and surgical management, including the use of collagen cross-linking (CXL).

However, as explored in Part IIA (chapter 5) the limitations in technology utilised in previous prospective longitudinal studies of the natural history of keratoconus may not be easily overcome in NZ with CXL becoming standard of care for progressive keratoconus. Thus this retrospective longitudinal investigation (ARK Part IIC) was developed to supplement the results of ARK Part IIB and further investigate the suggestion that keratoconus continues to progress beyond age 30. Yet this investigation was focussed on computerised corneal topography in subjects that have never worn...
CLs, thus eliminating the potentially confounding effects of CL wear on corneal shape/power and improving the accuracy of longitudinal measurements of cornea curvature/power by utilising analysis of computerised corneal topography.

**Aims**

The aims of this study were to determine;

1) If significant progression of manifest keratoconus occurs beyond age 30 in non-contact lens wearers, based on longitudinal changes in topographic parameters

2) If topographic disease severity and subject-specific parameters can be used to predict the likelihood of progression

**7.2 Methods**

**7.2.1 Study Protocol and Design**

Similar to the ARK Study: Part IIB in chapter 6, this study was a retrospective, longitudinal investigation. The clinical records of subjects with keratoconus who underwent consultation between 2002-2015 at a sub-specialty optometry practice with more than 40 years of specialised provision of eye care for keratoconus (Mortimer Hirst Eyecare and Eyewear, Auckland, New Zealand) (MH) were reviewed. The outcome measures for this study were obtained from the axial power maps of the Medmont-E300 (Medmont, Camberwell, Australia, Medmont studio version 6.1.1.4) as indicated in chapter 3, for the first (baseline) and most recent (final) reviews for all eyes of participants that met the selection criteria.

**7.2.2 Outcome Measures**

- Maximum corneal power ($K_{\text{max}}$)
- Steep simulated keratometry ($K_{\text{steep}}$)
- Flat simulated keratometry ($K_{\text{flat}}$)
- Inferior-superior dioptric asymmetry (I-S)$^{212}$
- Surface asymmetry index (SAI)$^{213}$
- Surface regularity index (SRI)$^{215}$

To determine the repeatability of these parameters obtained from the Medmont-E300 in patients with keratoconus, three consecutive scans were carried out on 20 randomly selected subjects with keratoconus that met the selection criteria of the study. However, CL wearers were included if no CL was worn for ≥2 days. The median and interquartile range, within subject standard deviation ($S_w$), precision (1.96$S_w$) and repeatability (2.77$S_w$) was calculated for all parameters.
7.2.3 Participants

The electronic and hard copy database of MH was analysed to identify potential participants. This investigation was focused on subjects with manifest keratoconus that did not have a history of contact lens wear, thus all subjects diagnosed with manifest keratoconus were identified and subject to the selection criteria described below.

Ethical approval to carry out this study was obtained from the University of Auckland Human Participant Ethics committee (UoAHPEC) as an amendment to the ethical approval obtained for the ARK Study: Part I, approval number: 010547.

Inclusion Criteria
- Manifest keratoconus. As stated in chapter 3, the diagnosis of keratoconus was made by a community optometrist, on the basis of examination utilising a combination of keratometry or corneal topography, slit-lamp biomicroscopy and refraction
- Age ≥30 years
- At least two consultations ≥12 months apart

Exclusion Criteria
- Previous/current episode of acute corneal hydrops in either eye
- Forme fruste keratoconus
- Any previous contact lens wear
- Previous ocular surgery (including keratoplasty and corneal collagen cross-linking (CXL)) or trauma
- Systemic disease which may affect the cornea (e.g. diabetes mellitus)
- Previous corneal herpetic disease
- Any other ocular pathology
**Statistical Analysis**

The Kolmogorov-Smirnov test revealed that all study parameters were non-parametric.

The difference for all outcome measures between baseline and final reviews, for all eyes, were compared utilising the Wilcoxon signed-rank test. Only parameters that demonstrated a significant change between reviews were utilised in subsequent analyses.

The Mann-Whitney U test was used to investigate differences in all outcome measures between the repeatability and main study groups to assess potential differences in disease severity between the groups. Thus this analysis was to determine the applicability of the repeatability measure in forming criteria for disease progression.

Eyes were subsequently grouped according to degree of progression based on the repeatability analysis of the topographic parameters as indicated in Table 7-1B; no progression (<1.00D change) and significant progression (≥1.00D increase). The rate of change in the variables that demonstrated statistically significant differences between baseline and final review was assessed and the number of eyes with significant rates of progression was determined, defined using similar criteria to progression; non-significant rate of progression (<1.00D change/year), and significant rate of progression (≥1.00D increase/year).

Univariate associations between potential predictors and changes in outcome measures were evaluated; Spearman correlation for continuous predictors and outcome measures, and Mann-Whitney U test for dichotomous predictors; gender, age at baseline and follow-up time. The Spearman correlation was also utilised to determine if the changes in outcome measures were correlated with each other.

Factors associated with an increase of ≥1.00D in at least one eye for all outcome measures were performed on a participant-specific basis, using the between-eye mean of eye-specific predictors, such as $K_{\text{max}}$ at baseline, in the analyses. The initial analyses included the Mann-Whitney U test for continuous predictors and the Chi square test for dichotomous predictors. All variables that were significant were then included in a logistic regression analysis to produce odds ratios (OR) and 95% confidence intervals (CI). Age at baseline and follow-up time were assessed as continuous and dichotomous predictors; age ≥40 and <40 years and follow-up time <5 and ≥5 years.

All analyses were performed using SPSS 22.0 for Windows (Chicago, IL, USA). A p-value of <0.05 was considered as significant throughout.
7.3 Results

Of the 449 potential participants with manifest keratoconus identified, 27 participants met the selection criteria and 43 eyes of these participants were included in the analyses. The median age of the cohort at baseline was 38.45 (12.86) years, 13 (48.1%) participants were ≥40 years old, and 14 (52.0%) participants were male. The median age at final review was 43.21 (14.86) years. The median follow-up time was 4.36 (8.68) years with 11 (40.7%) participants having ≥5 years follow-up.

All topographic parameters demonstrated a significant median increase between baseline and final review (p<0.05), except for SAI and SRI which did not change significantly (p>0.600) (Table 7-1A.). The repeatability analysis of the topographic parameters was conducted on 37 eyes of 20 subjects and suggests that a change of ≥1.00D is indicative of significant disease progression for all parameters except for SRI (Table 7-1B.). The median $K_{\text{max}}$, $K_{\text{steep}}$, and SRI were significantly higher in the repeatability cohort, compared to the study cohort.

Table 7-1 A. Change in computerised topographic parameters between baseline and final reviews in the main study group, 47 eyes of 27 participants; maximum keratometry ($K_{\text{max}}$), steep simulated keratometry ($K_{\text{steep}}$), flat simulated keratometry ($K_{\text{flat}}$), inferior-superior dioptic asymmetry (I-S), surface asymmetry index (SAI) and surface regularity index (SRI). All values are Median (interquartile range).

<table>
<thead>
<tr>
<th>A. Variable</th>
<th>Baseline</th>
<th>Final</th>
<th>Change in Parameter (Final – Baseline)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{\text{max}}$ (D)</td>
<td>50.91 (8.01)</td>
<td>52.00 (8.04)</td>
<td>0.30 (1.21)</td>
<td>0.039*</td>
</tr>
<tr>
<td>$K_{\text{steep}}$ (D)</td>
<td>46.64 (7.84)</td>
<td>47.36 (7.86)</td>
<td>0.27 (0.90)</td>
<td>0.003*</td>
</tr>
<tr>
<td>$K_{\text{flat}}$ (D)</td>
<td>44.66 (4.92)</td>
<td>44.96 (5.39)</td>
<td>0.34 (1.12)</td>
<td>0.003*</td>
</tr>
<tr>
<td>I-S (D)</td>
<td>2.90 (3.69)</td>
<td>3.50 (3.79)</td>
<td>0.26 (0.82)</td>
<td>0.004*</td>
</tr>
<tr>
<td>SAI</td>
<td>3.70 (4.89)</td>
<td>4.14 (4.64)</td>
<td>-0.09 (0.88)</td>
<td>0.628</td>
</tr>
<tr>
<td>SRI</td>
<td>1.16 (0.87)</td>
<td>1.13 (0.69)</td>
<td>-0.01 (0.19)</td>
<td>0.744</td>
</tr>
</tbody>
</table>

Table 7-1 B. Repeatability analysis of $K_{\text{max}}$, $K_{\text{steep}}$, $K_{\text{flat}}$, I-S, SAI and SRI on 37 eyes of 20 randomly selected subjects with keratoconus to determine criteria for progression.

<table>
<thead>
<tr>
<th>B. Variable</th>
<th>Median (IQ Range)</th>
<th>Within Subject Standard Deviation</th>
<th>Precision</th>
<th>Repeatability</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{\text{max}}$ (D)</td>
<td>54.59 (8.39)†</td>
<td>0.33</td>
<td>0.65</td>
<td>0.92</td>
</tr>
<tr>
<td>$K_{\text{steep}}$ (D)</td>
<td>51.61 (8.45)†</td>
<td>0.33</td>
<td>0.64</td>
<td>0.91</td>
</tr>
<tr>
<td>$K_{\text{flat}}$ (D)</td>
<td>46.43 (5.23)</td>
<td>0.34</td>
<td>0.66</td>
<td>0.94</td>
</tr>
<tr>
<td>I-S (D)</td>
<td>3.22 (3.69)</td>
<td>0.36</td>
<td>0.71</td>
<td>1.01</td>
</tr>
<tr>
<td>SAI</td>
<td>4.22 (4.21)</td>
<td>0.38</td>
<td>0.74</td>
<td>1.05</td>
</tr>
<tr>
<td>SRI</td>
<td>1.50 (0.65)†</td>
<td>0.09</td>
<td>0.17</td>
<td>0.24</td>
</tr>
</tbody>
</table>

* = statistically significant difference between baseline and final visit
† = statistically significant difference between parameter in repeatability group and main study group (p<0.05)
The median rate of change (D/year) was; 0.06 (0.28), 0.04 (0.19), 0.08 (0.15), and 0.04 (0.20), for $K_{\text{max}}$, $K_{\text{steep}}$, $K_{\text{flat}}$ and $I-S$, respectively. The majority of eyes (93.0%–100.0%) exhibited a non-significant rate of progression (<1.00D change/year) for all parameters. A significant rate of progression (≥1.00D/year) was observed in $K_{\text{max}}$ and $K_{\text{steep}}$, 7.0% and 2.3% of eyes, respectively (Figure 7-1A.).

The greatest proportion of eyes (74.4%–81.4%) exhibited no progression over the follow-up period, with an overall change <1.00D between baseline and final reviews for all topographic parameters. However, a total increase of ≥1.00D in corneal topographic parameters was observed in 18.6%–25.6% of eyes (Figure 7-1B.).
Table 7-2 illustrates associations between potential predictors and progression in computerised corneal topographic parameters. The change in $K_{\text{steep}}$ and $K_{\text{flat}}$ demonstrate significant positive correlations with follow-up time, while $K_{\text{max}}$ and $K_{\text{steep}}$ increased significantly in female participants. There were significant moderate-strong positive correlations between the changes in all variables; $r>0.4$, $p<0.01$ in all cases.

Table 7-2 Spearman correlations and univariate associations for change in computerised corneal topographic parameters, maximum keratometry ($K_{\text{max}}$), steep simulated keratometry ($K_{\text{steep}}$), flat simulated keratometry ($K_{\text{flat}}$), inferior-superior dioptic asymmetry (I-S); between baseline and final review, participant factors, and follow-up time

<table>
<thead>
<tr>
<th>Variable</th>
<th>$K_{\text{max}}$</th>
<th>$K_{\text{steep}}$</th>
<th>$K_{\text{flat}}$</th>
<th>I-S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at baseline review (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r = 0.087$</td>
<td>$r = -0.155$</td>
<td>$r = -0.103$</td>
<td>$r = 0.067$</td>
<td></td>
</tr>
<tr>
<td>$p = 0.581$</td>
<td>$p = 0.320$</td>
<td>$p = 0.511$</td>
<td>$p = 0.670$</td>
<td></td>
</tr>
<tr>
<td>Age category at baseline review (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40</td>
<td>0.22 (1.47)</td>
<td>0.16 (1.85)</td>
<td>0.70 (1.15)</td>
<td>0.15 (0.60)</td>
</tr>
<tr>
<td>$p = 0.905$</td>
<td>$p = 1.000$</td>
<td>$p = 0.981$</td>
<td>$p = 0.302$</td>
<td></td>
</tr>
<tr>
<td>≥40</td>
<td>0.32 (0.85)</td>
<td>0.29 (1.21)</td>
<td>0.41 (0.86)</td>
<td>0.32 (0.99)</td>
</tr>
<tr>
<td>Follow-up time (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r = 0.015$</td>
<td>$r = 0.039$</td>
<td>$r = 0.102$</td>
<td>$r = 0.118$</td>
<td></td>
</tr>
<tr>
<td>$p = 0.925$</td>
<td>$p = 0.804$</td>
<td>$p = 0.515$</td>
<td>$p = 0.453$</td>
<td></td>
</tr>
<tr>
<td>Follow time Category (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>0.30 (0.77)</td>
<td>-0.02 (0.51)</td>
<td>0.16 (0.84)</td>
<td>0.09 (0.54)</td>
</tr>
<tr>
<td>$p = 0.753$</td>
<td>$p = 0.026*$</td>
<td>$p = 0.017*$</td>
<td>$p = 0.089$</td>
<td></td>
</tr>
<tr>
<td>≥5</td>
<td>0.35 (1.71)</td>
<td>1.22 (1.69)</td>
<td>0.84 (1.11)</td>
<td>0.43 (1.94)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.07 (0.82)</td>
<td>-0.02 (0.89)</td>
<td>0.35 (0.88)</td>
<td>0.18 (1.21)</td>
</tr>
<tr>
<td>$p = 0.956$</td>
<td>$p = 0.043*$</td>
<td>$p = 0.280$</td>
<td>$p = 0.756$</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>0.66 (1.20)</td>
<td>1.17 (1.61)</td>
<td>0.74 (1.32)</td>
<td>0.32 (0.60)</td>
</tr>
<tr>
<td>$p = 0.007*$</td>
<td>$p = 0.043*$</td>
<td>$p = 0.017*$</td>
<td>$p = 0.089$</td>
<td></td>
</tr>
<tr>
<td>$K_{\text{max}}$ baseline (D)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r = -0.066$</td>
<td>$r = 0.108$</td>
<td>$r = 0.115$</td>
<td>$r = 0.102$</td>
<td></td>
</tr>
<tr>
<td>$p = 0.675$</td>
<td>$p = 0.493$</td>
<td>$p = 0.464$</td>
<td>$p = 0.517$</td>
<td></td>
</tr>
<tr>
<td>$K_{\text{steep}}$ baseline (D)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r = -0.079$</td>
<td>$r = -0.022$</td>
<td>$r = -0.035$</td>
<td>$r = -0.012$</td>
<td></td>
</tr>
<tr>
<td>$p = 0.572$</td>
<td>$p = 0.887$</td>
<td>$p = 0.822$</td>
<td>$p = 0.941$</td>
<td></td>
</tr>
<tr>
<td>$K_{\text{flat}}$ baseline (D)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r = 0.009$</td>
<td>$r = 0.087$</td>
<td>$r = -0.082$</td>
<td>$r = 0.078$</td>
<td></td>
</tr>
<tr>
<td>$p = 0.956$</td>
<td>$p = 0.581$</td>
<td>$p = 0.603$</td>
<td>$p = 0.630$</td>
<td></td>
</tr>
<tr>
<td>I-S baseline (D)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r = -0.71$</td>
<td>$r = 0.070$</td>
<td>$r = 0.135$</td>
<td>$r = -0.031$</td>
<td></td>
</tr>
<tr>
<td>$p = 0.665$</td>
<td>$p = 0.669$</td>
<td>$p = 0.405$</td>
<td>$p = 0.850$</td>
<td></td>
</tr>
<tr>
<td>Change in $K_{\text{max}}$ (D)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r = 0.490$</td>
<td>$r = 0.397$</td>
<td>$r = 0.510$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p = 0.001*$</td>
<td>$p = 0.008*$</td>
<td>$p &lt; 0.001*$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in $K_{\text{steep}}$ (D)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r = 0.641$</td>
<td>$r = 0.404$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p &lt; 0.001*$</td>
<td>$p = 0.007*$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in $K_{\text{flat}}$ (D)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| * = statistically significant result
Figure 7-2 and Table 7-2 highlight the relationship between $K_{\text{steep}}$ and follow-up time as a dichotomous variable. A greater proportion of eyes experienced no progression (<1.00D increase) in the group with <5 years follow-up (88.9% vs. 56.3% for <5 and ≥5 year follow-up, respectively), while a greater proportion of eyes with ≥5 year follow-up experienced significant progression (≥1.00D increase, 11.1% vs. 43.7% for <5 and ≥5 year follow-up, respectively).

Figure 7-2 Change in steep keratometry ($K_{\text{steep}}$) between baseline and final review A. 16 eyes of 11 participants with 5 years of follow-up or more B. 27 eyes of 16 participants with less than 5 years of follow-up
Table 7-3. shows the predictors for ≥1.00D increase in a topographic parameter in at least one eye. Follow-up time as a continuous variable and gender were significantly associated with $K_{\text{steep}}$ ($p=0.031$) and $K_{\text{max}}$ ($p=0.004$), respectively.

Table 7-3 Factors associated with ≥1.00D increase in at least 1 eye in outcome measures with significant change between baseline and final review

<table>
<thead>
<tr>
<th>Continuous Predictors</th>
<th>% of patients</th>
<th>≥1.00D Increase in at least 1 eye</th>
<th>$K_{\text{max}}$ (D)</th>
<th>$K_{\text{steep}}$ (D)</th>
<th>$K_{\text{flat}}$ (D)</th>
<th>I-S (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of patients</td>
<td>Yes</td>
<td>33.3%</td>
<td>37.0%</td>
<td>37.0%</td>
<td>18.5%</td>
<td>66.6%</td>
</tr>
<tr>
<td>No</td>
<td>66.6%</td>
<td>37.0%</td>
<td>37.0%</td>
<td>18.5%</td>
<td>66.6%</td>
<td>37.0%</td>
</tr>
<tr>
<td>Age at review 1 (years)</td>
<td>Yes</td>
<td>39.10 (23.42)</td>
<td>37.29 (14.68)</td>
<td>36.98 (14.12)</td>
<td>34.23 (20.96)</td>
<td>18.5%</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>37.29 (12.93)</td>
<td>39.11 (10.66)</td>
<td>41.54 (14.54)</td>
<td>39.11 (12.02)</td>
<td>63.0%</td>
</tr>
<tr>
<td>Follow-up time (years)</td>
<td>Yes</td>
<td>3.74 (9.13)</td>
<td>0.940</td>
<td>2.70 (2.63)</td>
<td>0.103</td>
<td>12.13 (9.22)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>4.37 (9.07)</td>
<td>14.68</td>
<td>2.89 (7.14)</td>
<td>0.232</td>
<td>3.82 (7.70)</td>
</tr>
<tr>
<td>$K_{\text{max}}$ baseline (D)</td>
<td>Yes</td>
<td>53.86 (11.41)</td>
<td>52.90 (4.85)</td>
<td>52.86 (7.05)</td>
<td>52.90 (5.56)</td>
<td>53.7</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>50.37 (5.76)</td>
<td>50.30 (8.46)</td>
<td>50.37 (7.02)</td>
<td>50.30 (7.35)</td>
<td>63.0%</td>
</tr>
<tr>
<td>$K_{\text{steep}}$ baseline (D)</td>
<td>Yes</td>
<td>50.12 (12.17)</td>
<td>48.00 (6.33)</td>
<td>47.15 (7.61)</td>
<td>47.64 (9.75)</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>46.30 (4.17)</td>
<td>46.82 (8.57)</td>
<td>46.74 (5.60)</td>
<td>47.15 (5.59)</td>
<td>18.5%</td>
</tr>
<tr>
<td>$K_{\text{flat}}$ baseline (D)</td>
<td>Yes</td>
<td>48.89 (7.66)</td>
<td>47.15 (7.61)</td>
<td>44.09 (6.08)</td>
<td>45.54 (8.90)</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>44.70 (2.70)</td>
<td>47.64 (7.34)</td>
<td>45.31 (4.70)</td>
<td>44.85 (4.55)</td>
<td>66.6%</td>
</tr>
<tr>
<td>I-S baseline (D)</td>
<td>Yes</td>
<td>3.26 (5.23)</td>
<td>3.00 (6.22)</td>
<td>3.00 (5.46)</td>
<td>3.00 (5.20)</td>
<td>18.5%</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>3.00 (3.33)</td>
<td>2.89 (3.65)</td>
<td>3.00 (3.87)</td>
<td>2.89 (4.04)</td>
<td>66.6%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Categorical Predictors</th>
<th>Category</th>
<th>% ≥1.00D in at least one eye</th>
<th>% ≥1.00D in at least one eye</th>
<th>% ≥1.00D in at least one eye</th>
<th>% ≥1.00D in at least one eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow time Category (years)</td>
<td>&lt;5</td>
<td>18.5</td>
<td>14.8</td>
<td>14.8</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>&gt;5</td>
<td>14.8</td>
<td>22.2</td>
<td>22.2</td>
<td>14.8</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>3.7</td>
<td>11.1</td>
<td>14.8</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>29.6</td>
<td>25.9</td>
<td>22.2</td>
<td>7.4</td>
</tr>
<tr>
<td>Age category at baseline review (years)</td>
<td>≤40</td>
<td>18.5</td>
<td>22.2</td>
<td>25.9</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>&gt;40</td>
<td>14.8</td>
<td>14.8</td>
<td>11.1</td>
<td>7.4</td>
</tr>
</tbody>
</table>

* = statistically significant result

The results of the logistic regression analysis demonstrate that follow-up time is a significant predictor for ≥1.00D increase in $K_{\text{steep}}$ in at least one eye (OR=1.38; 95%CI 1.04–1.84, $p=0.026$), while male gender was significantly associated with a reduction in the risk of ≥1.00D increase in $K_{\text{max}}$ in at least one eye (OR=0.048, 95%CI 0.005–0.490, $p=0.010$).
7.4 Discussion

As discussed in chapters 2, 4, 5 and 6, the pathogenesis, mechanisms and rate of progression of keratoconus are not completely understood. It has been noted that there is an increase in the amount of non-enzymatic cross-linking within the cornea as one ages\textsuperscript{262} likely due to exposure to ultraviolet radiation throughout life, which is a possible mechanism by which keratoconus progression is slowed or halted with increasing age.\textsuperscript{263} Additionally, the CLEK reported that fewer patients progressed to transplantation later in life (12\%–20\% aged 10–40 vs. 3\%–8\% >40) and that older age at baseline was protective against requiring transplantation (OR=0.72).\textsuperscript{264}

The current study assessed the progression of keratoconus utilising quantitative computerised corneal topography, and it is believed that this study is unique in that it is focussed on the natural history of subjects with keratoconus: A) that had never worn contact lenses, B) assessment focussed on computerised topography parameters at baseline and final review (median follow-up 4.36 (8.68) years) and C) that were typically a little older than those in other investigations of keratoconus. As previously mentioned, the lack of previous CL wear avoided the potentially confounding effects of CL wear on corneal shape and possibly the keratoconus disease process. The majority of variables investigated increased significantly between the baseline and final reviews ($K_{\text{max}}$ 0.30D, $K_{\text{steep}}$ 0.27D, $K_{\text{flat}}$ 0.34D and I-S 0.26D), suggesting that corneal curvature/power in subjects with keratoconus over age 30 continues to increase. Additionally, changes in each parameter had a moderate-strong positive correlation with the changes in all other parameters, thus an increase in one parameter may be accompanied by a similar increase in others, suggesting that all topographic parameters investigated could potentially be utilised to monitor progression.

The results of this study are somewhat comparable to the landmark CLEK, which prospectively tracked changes in corneal curvature over eight years in 1032 subjects with mean age 38.9 years at enrolment.\textsuperscript{253} Measured using manual keratometry, The CLEK reported that $K_{\text{flat}}$ increased at a mean rate of $0.18 \pm 0.60\text{D/year}$.\textsuperscript{253} However, as also noted in chapter 6, the authors of the CLEK suggested that progression is non-linear with the greatest rate of progression occurring earlier in life; the average rate of increase in $K_{\text{flat}}$ was 1.0D/year in the age group <20 years, decreasing to approximately 0.4D/year in the age group 20–29 years, 0.2D/year in the age group 30–39 years and 0.1D/year in the age group ≥40 years.\textsuperscript{253} The median rates of change in our cohort (median age 38.5 years), were between 0.06–0.08D/year for all outcome measures, similar to that reported in the CLEK for patients aged ≥40. Notably, over 90\% of eyes in our study cohort had <1.00D change/year, suggesting that any change in corneal curvature/power that occurs later in life in subjects with keratoconus occurs at a slow pace in most cases.
An increase of ≥3.00D in $K_{\text{flat}}$ over the duration of the CLEK was considered significant and occurred in 39.1% of patients aged <30 years, but just 19.8% aged ≥30. The authors of the CLEK hypothesised that the natural history of keratoconus is dichotomous; the majority of progression occurring before age 35, which revealed that patients <35 years of age are more likely to have an increase of ≥3.00D in $K_{\text{flat}}$ in at least one eye (OR=3.12). Interestingly, in the current study using computerised corneal topographic measures, 21% of eyes in this older cohort demonstrated an increase in $K_{\text{max}}$ approaching 3.00D.

The Medmont-E300 has been shown to be highly repeatable on normal corneas, however, unlike other topography instruments, it has not been verified on keratoconic corneas. The surface irregularity of keratoconic corneas has been demonstrated to reduce the repeatability of topography devices. The current investigation performed a repeatability analysis on keratoconic corneas in order to produce categorical criteria for progression and found that ≥1.00D increase in parameters indicated significant progression. A recent investigation of the natural history of corneal curvature with increasing age in normal subjects, utilising the Medmont-E300, found no trend toward change in corneal curvature up to age 69. Thus utilisation of the repeatability to form criteria for progression in eyes with keratoconus is justified.

The criterion for progression (≥1.00D increase) in this study was robust as the repeatability group had a greater average $K_{\text{max}}$, which may reduce the repeatability of topography devices, thus, the change required for progression to be detected in the main study group was in fact likely to be less than a 1.00D increase. The current investigation elucidated that approximately 20% of eyes underwent significant progression (≥1.00D increase in a parameter) and 80% no progression (<1.00D increase in a parameter), while a third of participants experienced significant progression in at least one eye. While the changes in the anterior topographical parameters documented in this investigation have been verified as a means of detecting progression, recent evidence suggests that changes in the posterior surface may occur prior to the anterior surface. The Placido-based Medmont-E300 does not enable evaluation of the posterior corneal surface or corneal thickness, thereby limiting the definition of progression utilised in this study to changes in anterior topographical parameters.

The characteristics of subjects with keratoconus and risk factors for progression have been established by prospective observational studies such as CLEK and the Dundee University of Keratoconus study (DUSKS). The CLEK in particular established that the greatest predictors for progression were younger age and greater disease severity at baseline. However, as previously noted, the CLEK quantified corneal shape by keratometry, not computerised corneal topography, and
a large number of participants wore rigid CLs which may have caused corneal distortion/warpage. In the current study, the only significant predictor of disease progression was follow-up time; therefore it is possible that given enough time, additional subjects in the study cohort may experience significant progression. However, the large range of follow-up durations poses a limitation in estimations of rate of progression, as theoretically, eyes with longer follow-up duration might have experienced progression following the baseline visit but progression had ceased before the final visit, producing an artificially low average rate of progression, while there may in fact have been a significant rate of progression over some of the follow-up period.

In the current study male gender appears to be protective against ≥1.00D increase in $K_{\text{max}}$ in at least one eye (OR=0.048) though this may reflect a limitation of the sample size. Additionally, all baseline topographic parameters were higher in the group with ≥1.00D change in at least one eye at baseline, implicating greater disease severity as a potential predictor of progression, despite none of these baseline parameters being statistically significant. Once more this may be a limitation of the sample size as a number of prior studies have reported steeper keratometry to be a significant predictor of progression.271, 272

The sample size in the current study was limited largely due to strict inclusion/exclusion criteria limiting it to subjects with keratoconus that had never worn CLs; generally these are subjects with mild-moderate rather than severe disease. Indeed, presuming that disease severity may be a predictor for progression, subjects with greater disease severity may have already progressed to CL wear or even corneal transplantation by this age, so it would be difficult/impossible to accurately assess disease progression by computerised topography. In this respect it is notable that of 449 subjects with keratoconus assessed for this study, 94% had worn contact lenses, had developed corneal scarring or had progressed to corneal transplantation and only 6% of subjects met the inclusion criteria. Changes in visual acuity, spectacle refraction and the effect on progression of commonly known, non-corneal topographic, associations of keratoconus including family history, eye rubbing and atopy could not be accurately investigated due to variations in recorded history, spectacle prescriptions occasionally being recorded without an associated VA, and the occasional provision of reduced cylindrical correction to maximise spectacle tolerance.
7.5 Conclusions

Overall, this study highlights that keratoconus may continue to progress in apparently clinically stable non-CL wearing subjects beyond the fourth decade of life, however, <10% of eyes may progress at a rate of ≥1.00D/year. These data suggest that subjects with keratoconus, over the age of 30 years, should still be monitored for progression long term, as on average one in three may experience significant progression in at least one eye, though this proportion might be higher in subjects with more severe disease and those with longer follow-up. Indeed, 21% of eyes exhibited a mean increase of 2.73D in K_{max} over the duration of the study. This has significant implications for the consideration of CXL, particularly in older subjects with keratoconus progressing at a significant rate, as well as the use of toric intra-ocular lenses in the context of cataract surgery in keratoconus.\textsuperscript{228} However, CXL may not yield a significant benefit in patients with very slowly progressing disease.
Section 4:

Repeatability and Agreement of Computerised Corneal Tomography Devices and Accelerated Corneal Collagen Cross-linking

Chapters 8-9
Chapter 8:

Repeatability and Agreement of Orbscan II, Pentacam HR and Galilei G2 Tomography Systems in Corneas with Keratoconus - The Aotearoa Research into Keratoconus (ARK) Study: Part IIIA
8.1 Introduction

As mentioned in previous chapters, keratoconus is a disease characterised by steepening and thinning of the cornea, thus accurate and precise measurements of the cornea are required for the diagnosis and monitoring of keratoconus. Measurements of corneal curvature/power and thickness are most frequently used to make the diagnosis of keratoconus and to monitor for progression of the disease. In addition, some treatment options, including corneal collagen cross-linking (CXL) and intracorneal ring segments, require accurate pachymetry measurements to determine whether these procedures can be safely performed.

There are several commercially available instruments for the measurement of corneal curvature and thickness which differ in the technology used. As detailed in chapter 3, the Orbscan II (Bausch & Lomb, Rochester, NY) uses slit-scanning and Placido disc technology to combine corneal curvature/power measurements with assessment of the anterior and posterior corneal surfaces which allows a three dimensional reconstruction of the cornea. Additionally, chapter 3 described the Pentacam HR system (Oculus, Wetzlar, Germany), which uses a single rotating Scheimpflug camera and monochromatic slit-light source in combination with a static camera to obtain multiple slit images which correspond to specific angles along the optical axis. Finally, chapter 3 also described the Galilei G2 (Ziemer Ophthalmology Co. Allmendstrasse, Switzerland), which combines dual rotating Scheimpflug cameras and a Placido disc to assess the anterior segment.

Our research group previously reported on repeatability and agreement of these three instruments when measuring healthy corneas. However, there are a paucity of data describing the performance of these instruments when measuring corneas that significantly deviate from normal parameters such as keratoconic corneas.

This study was conceived following the initial recruitment of 7 participants into the investigation of CXL detailed in chapter 9, as these participants had initially been examined using the Orbscan II (with an acoustic factor of 0.92 for corneal thickness measurements) prior to being referred to the specialty CXL clinic. It was immediately noted that these pachymetry measurements were significantly lower than those obtained with the Pentacam HR, which prompted the author to investigate the repeatability and comparability of the computerised corneal tomography devices available at the University of Auckland clinical research laboratory located within the Ophthalmology Department at Greenlane Clinical Centre (GLCC), ADHB, Auckland, New Zealand.
Aims

The aims of this study were to;

1) Assess the repeatability of corneal curvature/power and pachymetry measurements obtained using the Orbscan II, Pentacam HR and Galilei G2 computerised corneal tomographers in eyes with keratoconus

2) Assess the agreement of corneal curvature/power and pachymetry measurements obtained using the Orbscan II, Pentacam HR and Galilei G2 computerised corneal tomographers in eyes with keratoconus

8.2 Methods

8.2.1 Study Protocol and Design

This study was a prospective, comparative investigation. Subjects with keratoconus were evaluated utilising three computerised corneal tomographic devices; the Orbscan II, Pentacam HR, and Galilei G2. All three tomographers were calibrated prior to beginning the study. No eye drops were applied prior to testing. Three measurements were taken per eye using each of the three devices as per the methodology outline in chapter 3. The devices were utilised in a random order for each analysed eye. For each subject, all measurements were performed within a 30-minute time period. One eye from each subject was selected randomly unless only one eye met the selection criteria.

As mentioned in chapter 3, both the Pentacam HR and Galilei G2 provide a “quality score” of individual measurements. The Pentacam HR provides a quality specification of “OK” if the scan is of acceptable quality. The Galilei breaks down the quality percentage of the image into four components: motion compensation, Placido, Scheimpflug, and motion distance. These components are then summarised as an overall quality score along with a reference minimum required percentage score. The Orbscan II does not provide a quality score but instead automatically discards measurements deemed to be of unacceptable quality. Where specifically noted, an acoustic correction factor of 0.92 was applied to Orbscan II data. Up to six total scans were attempted, per device, if the quality of any of the first three scans was <85% by Galilei, not deemed “OK” by Pentacam HR, or deemed of poor quality on Orbscan II.

All research procedures were carried out within the University of Auckland clinical research laboratory located within the Ophthalmology Department at GLCC, ADHB, Auckland, New Zealand.
8.2.2 Outcome Measures

- Steep simulated keratometry ($K_{steep}$)
- Flat simulated keratometry ($K_{flat}$)
- Central corneal thickness (CCT)
- Thinnest corneal thickness (TCT)
- Corneal densitometry (gray scale units) (GSU)

8.2.3 Participants

Participants were recruited from cornea and anterior segment sub-specialist clinics at the Ophthalmology service within GLCC, ADHB. Potential participants were referred internally to a specialist keratoconus and CXL treatment clinic, from which eligible participants, that also form most of the cohort in the investigation of CXL in chapter 9, were included in this study.

This investigation adhered to the tenets of the Declaration of Helsinki and ethical approval for the study was obtained from the Northern X Ethics division of the Health and Disability Ethics Committee (HDEC) of NZ, approval reference: NTX/08/08/070AM02.

Inclusion Criteria

- Manifest keratoconus. A diagnosis of keratoconus was made as per the criteria indicated in chapter 3, section 3.4.5
- Age >14 years at enrolment
- The ability to understand the implications of the study, such that the participant was able to provide fully informed written consent or a parent/guardian was able to do so if the participant was less than 16 years of age

Exclusion Criteria

- Previous/current episode of acute corneal hydrops in either eye
- Forme fruste keratoconus
- Previous contact lens wear
- Previous ocular surgery (including keratoplasty and corneal collagen cross-linking (CXL)) or trauma
- Systemic disease which may affect the cornea (e.g. diabetes mellitus)
- Previous corneal herpetic disease
- Any other ocular pathology
- Corneal scarring or oedema visible on slit-lamp examination
Statistical Analysis

The Kolmogorov-Smirnov test was performed to confirm that all study parameters were parametric.

Intraobserver repeatability was assessed using within-subject standard deviation ($S_w$), precision, intrasession test-retest variability (repeatability), coefficient of variation (CV), and intraclass correlation coefficient (ICC). Precision was calculated as $1.96S_w$, since for 95% of observations the difference between a subject’s measurement and the true value would be expected to be less than $1.96S_w$. Test-retest variability, or repeatability, was calculated as $2.77S_w$. The within-subject CV was calculated as the $S_w$ divided by the overall mean and expressed as a percentage. The ICC is defined as the ratio of the between-subjects variance to the sum of the combined within-subjects and between-subjects variance. The ICC values range from 0 to 1, with 1 indicating perfect agreement.

Bland-Altman plots were used to compare measurements between device pairs by plotting the differences between measurements against their mean along with constructs of the limits of agreement (LoA). The 95% LoA (mean difference ± 1.96 standard deviation) define the range within which most differences between measurements from the two devices will lie.

The Pearson correlation was utilised to determine if there was a correlation between corneal densitometry and within-subject standard deviation for the pachymetry and corneal curvature/power measurements. The Pearson correlation was also utilised to determine if there was a correlation between the corneal densitometry values and differences in measurements between instrument pairs for pachymetry and corneal curvature/power measurements.

All analyses were performed using SPSS 22.0 for Windows (Chicago, IL, USA). A p-value of <0.05 was considered as significant throughout.
8.3 Results

Fifty consecutive eyes (25 Right, 25 Left) of fifty participants were assessed and included in the analyses. The mean age was 23.3 ± 7.7 years (31 males, 19 females). Eyes were not excluded based on the quality of the scans. However, 12 eyes had at least one scan with a quality score below 85% as measured by the Galilei G2 and 6 eyes had at least one scan not deemed “OK” by Pentacam HR. Using the Pentacam HR classification of keratoconus severity; 5 eyes were grade I, 6 eyes grade I-II, 11 eyes grade II, 9 eyes grade II-III, 14 eyes grade III and 5 eyes Grade III-IV.

8.3.1 Repeatability of Corneal Curvature/Power Measurements

Repeatability parameters for $K_{\text{steep}}$ and $K_{\text{flat}}$ are summarized in Table 8-1. The Pentacam HR showed the highest repeatability and Orbscan II the lowest repeatability of $K_{\text{steep}}$ and $K_{\text{flat}}$ measurements. The ICC was greater than 0.98 for all devices.

8.3.2 Repeatability of Pachymetry Measurements

Repeatability parameters for CCT and TCT are summarized in Table 8-1. The Galilei G2 produced the lowest $S_W$ and CV, and the highest ICC, indicating a higher degree of repeatability of CCT and TCT compared to the Pentacam HR and Orbscan II. The Orbscan II showed the lowest degree of repeatability. The ICC was 0.98 or higher for all devices.
Table 8-1 Intraobserver repeatability for corneal curvature/power and pachymetry measurements obtained with the Pentacam HR, Galilei G2 and Orbscan II; steep simulated keratometry (K\text{\textsubscript{st}}), flat simulated keratometry (K\text{\textsubscript{fl}}), central corneal thickness (CCT) and thinnest corneal thickness (TCT).

CCT and TCT from Orbscan II assessed without (No AF) and with (0.92 AF) an acoustic factor of 0.92

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD (range)</th>
<th>Within Subject SD</th>
<th>Precision</th>
<th>Repeatability</th>
<th>CV (%)</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>K\text{\textsubscript{st}} (D)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentacam HR</td>
<td>50.31 ± 4.24 (43.27 – 59.20)</td>
<td>0.36</td>
<td>0.71</td>
<td>1.00</td>
<td>0.54</td>
<td>0.997</td>
</tr>
<tr>
<td>Galilei</td>
<td>50.50 ± 4.37 (43.43 – 59.50)</td>
<td>0.65</td>
<td>1.27</td>
<td>1.80</td>
<td>0.86</td>
<td>0.992</td>
</tr>
<tr>
<td>Orbscan II</td>
<td>50.28 ± 4.78 (42.53 – 61.97)</td>
<td>0.81</td>
<td>1.59</td>
<td>2.25</td>
<td>1.19</td>
<td>0.991</td>
</tr>
<tr>
<td><strong>K\text{\textsubscript{fl}} (D)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentacam HR</td>
<td>45.30 ± 3.11 (40.70 – 52.07)</td>
<td>0.32</td>
<td>0.63</td>
<td>0.90</td>
<td>0.53</td>
<td>0.996</td>
</tr>
<tr>
<td>Galilei</td>
<td>45.69 ± 3.26 (40.25 – 52.53)</td>
<td>0.59</td>
<td>1.16</td>
<td>1.64</td>
<td>0.80</td>
<td>0.989</td>
</tr>
<tr>
<td>Orbscan II</td>
<td>44.80 ± 3.03 (39.77 - 51.77)</td>
<td>0.59</td>
<td>1.16</td>
<td>1.64</td>
<td>0.99</td>
<td>0.987</td>
</tr>
<tr>
<td><strong>CCT (µm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentacam HR</td>
<td>479.18 ± 36.97 (371.33 – 549.67)</td>
<td>4.84</td>
<td>9.48</td>
<td>13.39</td>
<td>0.86</td>
<td>0.994</td>
</tr>
<tr>
<td>Galilei</td>
<td>479.15 ± 36.94 (373.00 – 547.33)</td>
<td>3.59</td>
<td>7.04</td>
<td>9.95</td>
<td>0.52</td>
<td>0.997</td>
</tr>
<tr>
<td>Orbscan II (No AF)</td>
<td>484.29 ± 57.24 (355.80 – 597.83)</td>
<td>12.11</td>
<td>23.73</td>
<td>33.54</td>
<td>2.08</td>
<td>0.985</td>
</tr>
<tr>
<td>Orbscan II (0.92 AF)</td>
<td>445.55 ± 52.66 (327.33 – 550.00)</td>
<td>11.14</td>
<td>21.83</td>
<td>30.85</td>
<td>2.06</td>
<td>0.985</td>
</tr>
<tr>
<td><strong>TCT (µm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentacam HR</td>
<td>453.89 ± 40.73 (360.00 – 538.67)</td>
<td>10.13</td>
<td>19.85</td>
<td>28.05</td>
<td>1.26</td>
<td>0.979</td>
</tr>
<tr>
<td>Galilei</td>
<td>463.35 ± 37.79 (368.00 – 538.67)</td>
<td>4.77</td>
<td>9.34</td>
<td>13.21</td>
<td>0.75</td>
<td>0.995</td>
</tr>
<tr>
<td>Orbscan II (No AF)</td>
<td>459.51 ± 61.21 (331.88 – 589.13)</td>
<td>13.12</td>
<td>25.72</td>
<td>36.34</td>
<td>2.51</td>
<td>0.985</td>
</tr>
<tr>
<td>Orbscan II (0.92 AF)</td>
<td>422.75 ± 56.31 (305.33 – 542.00)</td>
<td>12.07</td>
<td>23.66</td>
<td>33.44</td>
<td>2.52</td>
<td>0.985</td>
</tr>
</tbody>
</table>

8.3.3 Agreement of Corneal Curvature/Power Measurements

For the K\text{\textsubscript{st}} measurements, there were no significant differences in the mean values between device pairs. The mean K\text{\textsubscript{fl}} values were significantly different between all device pairs, the highest measurements obtained by the Galilei G2, followed by the Pentacam HR then Orbscan II. Bland-Altman plots for agreement of corneal curvature/power measurements are shown in Figure 8-1 and described in Table 8-2.
8.3.4 Agreement of Pachymetry Measurements

A comparison of CCT and TCT values recorded by the three devices is summarized in Table 8-2. Mean CCT measurements were not significantly different between instrument pairs. Mean TCT measurements were significantly higher for Galilei G2 compared to Pentacam HR. However, the Galilei G2 and Pentacam had the closest 95% limits of agreement for both CCT and TCT. Bland-Altman plots demonstrate the agreement of CCT and TCT between devices (Figure 8-1).

When an acoustic factor (0.92) was applied to Orbscan II pachymetry values, mean CCT measurements were significantly lower than Pentacam HR (-33.63µm; p<0.001, 95% limits of agreement: -79.25 to 11.98) and Galilei (-33.60µm; p<0.001, 95% limits of agreement: -74.59 to 7.39). Similarly, acoustic factor-adjusted, Orbscan II TCT measurements were significantly lower compared to Pentacam (-31.14µm; p<0.001, 95% limits of agreement: -76.41 to 14.13) and Galilei (-39.93µm; p<0.001, 95% limits of agreement: -95.87 to 16.00) (Table 8-2).

Table 8-2 Agreement of measurements of corneal curvature/power and pachymetry obtained with the Pentacam HR, Galilei G2 and Orbscan II; steep simulated keratometry (Ksteep), flat simulated keratometry (Kflat), central corneal thickness (CCT) and thinnest corneal thickness (TCT). CCT and TCT from Orbscan II assessed without (No AF) and with (AF) an acoustic factor of 0.92

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Agreement</th>
<th>Mean Difference</th>
<th>P-Value</th>
<th>Fixed Bias</th>
<th>Proportional Bias</th>
<th>95% LoA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ksteep (D)</td>
<td>Orbscan-Galilei</td>
<td>-0.18</td>
<td>0.477</td>
<td>No</td>
<td>No</td>
<td>-3.59 to 3.24</td>
</tr>
<tr>
<td></td>
<td>Orbscan-Pentacam</td>
<td>-0.05</td>
<td>0.792</td>
<td>No</td>
<td>Yes</td>
<td>-2.47 to 2.38</td>
</tr>
<tr>
<td></td>
<td>Galilei-Pentacam</td>
<td>0.13</td>
<td>0.611</td>
<td>No</td>
<td>No</td>
<td>-3.39 to 3.65</td>
</tr>
<tr>
<td>Kflat (D)</td>
<td>Orbscan-Galilei</td>
<td>-0.89</td>
<td>&lt;0.001*</td>
<td>Yes</td>
<td>No</td>
<td>-3.21 to 1.42</td>
</tr>
<tr>
<td></td>
<td>Orbscan-Pentacam</td>
<td>-0.50</td>
<td>&lt;0.001*</td>
<td>Yes</td>
<td>No</td>
<td>-2.14 to 1.15</td>
</tr>
<tr>
<td></td>
<td>Galilei-Pentacam</td>
<td>0.40</td>
<td>0.006*</td>
<td>Yes</td>
<td>No</td>
<td>-1.53 to 2.33</td>
</tr>
<tr>
<td>CCT (µm)</td>
<td>OrbscanNoAF-Galilei</td>
<td>5.11</td>
<td>0.153</td>
<td>No</td>
<td>Yes</td>
<td>-43.70 to 53.91</td>
</tr>
<tr>
<td></td>
<td>OrbscanNoAF-Pentacam</td>
<td>5.07</td>
<td>0.191</td>
<td>No</td>
<td>Yes</td>
<td>-47.95 to 58.09</td>
</tr>
<tr>
<td></td>
<td>OrbscanAF-Galilei</td>
<td>-33.60</td>
<td>&lt;0.001*</td>
<td>Yes</td>
<td>Yes</td>
<td>-74.59 to 7.39</td>
</tr>
<tr>
<td></td>
<td>OrbscanAF-Pentacam</td>
<td>-33.63</td>
<td>&lt;0.001*</td>
<td>Yes</td>
<td>Yes</td>
<td>-79.25 to 11.98</td>
</tr>
<tr>
<td></td>
<td>Galilei-Pentacam</td>
<td>-0.03</td>
<td>0.985</td>
<td>No</td>
<td>No</td>
<td>-25.26 to 25.26</td>
</tr>
<tr>
<td>TCT (µm)</td>
<td>OrbscanNoAF-Galilei</td>
<td>-3.20</td>
<td>0.490</td>
<td>No</td>
<td>Yes</td>
<td>-67.13 to 60.72</td>
</tr>
<tr>
<td></td>
<td>OrbscanNoAF-Pentacam</td>
<td>5.59</td>
<td>0.152</td>
<td>No</td>
<td>Yes</td>
<td>-47.62 to 58.80</td>
</tr>
<tr>
<td></td>
<td>OrbscanAF-Galilei</td>
<td>-39.93</td>
<td>&lt;0.001</td>
<td>Yes</td>
<td>Yes</td>
<td>-95.87 to 16.00</td>
</tr>
<tr>
<td></td>
<td>OrbscanAF-Pentacam</td>
<td>-31.14</td>
<td>&lt;0.001</td>
<td>Yes</td>
<td>Yes</td>
<td>-76.41 to 14.13</td>
</tr>
<tr>
<td></td>
<td>Galilei-Pentacam</td>
<td>8.79</td>
<td>&lt;0.001</td>
<td>Yes</td>
<td>No</td>
<td>-21.52 to 39.11</td>
</tr>
</tbody>
</table>

* Significant result (p<0.05)
Figure 8-1 Bland-Altman plots showing agreement in steep simulated keratometry ($K_{steep}$), flat simulated keratometry ($K_{flat}$), central corneal thickness (CCT) and thinnest corneal thickness (TCT) measurements obtained with the Pentacam HR, Galilei G2 and Orbscan II. Central line represents mean difference between the two devices. Dotted lines represent 95% limits of agreement.
8.3.5 Corneal densitometry measurements

Mean corneal densitometry measurements taken by Pentacam HR were 14.94 ± 1.50 gray scale units (range, 12.13 – 18.63), Sw 0.48, CV 3.61%, ICC 0.965. There were no significant correlations between densitometry values and within-subject standard deviation for the pachymetry or corneal curvature/power values measured by Pentacam HR, Galilei G2, or Orbscan II. There were also no significant correlations between densitometry values and differences in measurements between instrument pairs for pachymetry or corneal curvature/power.

8.4 Discussion

As more instruments for measuring anterior segment parameters become available, the comparability of measurements obtained using newer and existing technologies must be determined for healthy and pathologic eyes. By comparing instruments, it can be determined whether there is sufficient agreement for measurements from different devices to be used interchangeably, or directly compared. The repeatability of instruments must also be established to determine how much of a deviation from normal, or average, represents true pathology and the magnitude of change required to be considered progression of disease rather than deviation due to test variance.

This study examined the repeatability and comparability of three widely used instruments, the Orbscan II, Pentacam HR, and Galilei G2 corneal tomography systems in eyes with keratoconus. In comparison to the results from a previous study of healthy, non-keratoconic corneas,276 there was more variability (based on CV) in corneal curvature/power and CCT measurements of keratoconic corneas. The Orbscan II demonstrated lower repeatability compared to the Pentacam HR and Galilei G2 for all parameters investigated. The Pentacam HR showed the highest repeatability for corneal curvature/power measurements while the Galilei showed the highest repeatability for pachymetry measurements.

The results from this study suggest that the corneal curvature/power measurements of the investigated devices cannot be used interchangeably. The mean $K_{\text{flat}}$ values were significantly different between all instrument pairs. In contrast, mean $K_{\text{flat}}$ values were not significantly different when these instruments were previously studied in healthy, non-ectatic corneas,276 suggesting that image quality or ectasia may affect these measurements more than differences in hardware, software, or image acquisition between instruments. While the mean $K_{\text{steep}}$ values were similar between the instrument pairs, the limits of agreement were wider than what would be clinically acceptable.
Few previous studies have examined the repeatability of tomographic measurements in keratoconic eyes.\textsuperscript{207, 279-282} Some of these studies reported $K_{\text{mean}}$ values and did not specifically report on $K_{\text{steep}}$ or $K_{\text{flat}}$ measurements. However, the $K_{\text{steep}}$ and $K_{\text{flat}}$ measurements are of importance when diagnosing keratoconus or monitoring for progression. In addition, some of these studies did not specify whether eyes were excluded based on the quality of the scans obtained.

The mean CCT measurements were similar between Pentacam HR, Galilei G2 and Orbscan II. However, there were wide 95\% limits of agreement (spanning over 90µm) for Orbscan II measurements compared to Pentacam HR and Galilei G2. This indicates that, for some eyes, the CCT and TCT measurements taken by Orbscan II showed very poor agreement with Galilei and Pentacam HR.

The repeatability of pachymetry and corneal curvature/power measurements has importance when determining whether there has been progression in keratoconus. Test measurements with poor reproducibility make the detection of true progression more challenging due to difficulty in separating the test-retest variability from true change in measurements. Based on the results of this study, the higher test-retest variability of the Orbscan II measurements would require a significantly higher threshold for change to be considered genuine progression compared to Pentacam HR and Galilei.

Differences in the test-retest variability between instruments may be due to differences in technology used, such as different optical principles and light sources utilized by the devices.\textsuperscript{276} However, other factors may also account for these differences. In particular, the determination of acceptable scan quality may be a major factor. While Galilei G2 and Pentacam HR provide detailed information regarding the quality of scans, Orbscan II provides little information and automatically discards measurements deemed to be of unacceptable quality.

Contact ultrasound has often been referenced as the standard to which newer modalities have been compared. However, there are significant limitations in using contact ultrasound as a reference including: different operating modes of various ultrasounds, probe design and sensitivity, effects of the contact procedure (topical anaesthetic and corneal indentation), non-perpendicular probe position, and variability in the exact location of corneal measurement.\textsuperscript{283} Measurements of CCT obtained with the Pentacam HR have been shown to have higher repeatability and interexaminer correlation compared to ultrasound pachymetry for eyes with keratoconus.\textsuperscript{284}
Orbscan slit-scanning pachymetry has been compared to ultrasound in many studies, with variable and limited agreement. Overall, the Orbscan has been found to yield higher readings for corneal thickness (approximately 7% higher) compared to ultrasound. As a result, an acoustic (correction) factor of 0.92 was introduced to better align Orbscan pachymetry measurements with those obtained by ultrasound. The Orbscan software may incorporate this factor automatically as the default output or allow an alternative factor to be used, and clinicians should be aware of this setting.

Application of the acoustic factor to Orbscan measurements resulted in pachymetry values much lower than both Pentacam HR and Galilei G2. It has previously also been shown that for eyes with keratoconus, Orbscan II measurements (AF corrected) underestimate CCT compared to ultrasound. This has clinical implications as some eyes may not qualify for treatments such as corneal crosslinking based on adjusted pachymetry data from Orbscan II, but would qualify without the acoustic factor applied. In this study, Orbscan pachymetry data more closely correlated with Pentacam HR and Galilei G2 when the acoustic factor was not applied. Because the acoustic factor of 0.92 was derived using normal corneas, this factor may not be accurate when measuring ectatic corneas.

The Orbscan II uses optical pachymetry and relies on reflections and a clear optical pathway to obtain precise measurements. It has been shown that corneal haze can affect the scanning light system of Orbscan II, resulting in pachymetry measurements that are lower than those obtained using ultrasound. One possible explanation for the differences in pachymetry measurements between Orbscan II and Pentacam HR or Galilei G2 could be the presence of subclinical corneal haze or opacification. To test this hypothesis, we tested for any correlations between subclinical corneal opacification (as measured by corneal densitometry) and variability in measurements (corneal curvature/power and pachymetry) within devices. We also tested for any correlations between corneal densitometry and the magnitude of differences in measurements between devices. We did not detect any significant correlations, suggesting that degree of corneal clarity (as measured by Pentacam densitometry scores) was not a significant factor affecting the variability of measurements or differences between devices.

There are several possible limitations of this study. Inter-session and inter-examiner variability were not investigated and would be expected to also contribute to testing variability. Additionally, because measurements may be more variable with more severe keratoconus, the findings from this study may not be applicable to other cohorts of keratoconic eyes with more or less severe disease. However, the eyes included in this study had a wide range of severity of keratoconus.
Another limitation is the inclusion of some eyes with suboptimal scan quality. We did not exclude any eyes, even those with lower quality scans, in order to provide data more representative of measurements obtained in a typical clinical setting. When a higher cut-off of 85% for the quality score was used with the Galilei G2, analysis (not shown) demonstrated less variability in the measurements as would be expected. In practice, it is often difficult to obtain high quality scans of some eyes with severe keratoconus regardless of the number of attempted scans. Some of the reasons for inability to obtain an acceptable quality score include: severe disease causing difficulty obtaining alignment, poor patient fixation, blinking, dry eyes, or other corneal irregularity. It has been demonstrated that performing more measurements/scans within a session increases the sensitivity to detect change between testing sessions.281 We chose to perform analysis on three scans because this is a reasonable number of scans that could be performed in clinical practice. To limit any influence of the normal diurnal variations of corneal curvature/power and pachymetry,209 all measurements were performed within a 30 minute time period.

8.5 Conclusions
The results of this study suggest that the corneal curvature/power ($K_{\text{steep}}$ and $K_{\text{flat}}$) and pachymetry (CCT and TCT) measurements obtained with the Pentacam HR, Galilei G2 and Orbscan II cannot be used interchangeably between these tomographers in eyes with keratoconus. There are differences in repeatability of measurements taken with all three devices, with Orbscan II showing the lowest repeatability. These findings have clinically relevant implications when using different tomographers for the diagnosis, monitoring, and treatment of corneal ectatic disease such as keratoconus. In particular, determining criteria for the progression of keratoconus and which subjects may be eligible to safely undergo CXL or the insertion of intracorneal ring segments.
Chapter 9:

Safety and Efficacy of High Intensity, High Irradiance Accelerated Corneal Collagen Cross-linking with Continuous and Pulsed Ultraviolet Exposure - The Aotearoa Research into Keratoconus (ARK) Study: Part IIIIB
9.1 Introduction

Chapters 4-8 have focused on investigating the characteristics of keratoconus in NZ. The focus of the thesis in this chapter now shifts to the treatment of keratoconus utilising accelerated corneal collagen cross-linking.

As previously noted, in 2003 Wollensak et al. published a landmark non-randomised pilot clinical study detailing the procedure for corneal collagen crosslinking (CXL) as a means to halt or slow down the progression of keratoconus. While the natural history of keratoconus is not as yet completely understood (chapters 4-7), the intervening decade has witnessed the CXL procedure become increasingly recognized as an intervention with the potential to significantly modify the natural history of keratoconus. Known as the ‘Dresden protocol’, the original procedure requires removal of the central corneal epithelium, followed by application of photosensitizing riboflavin (vitamin B2) solution for 30 minutes and finally irradiation with ultraviolet-A (UV-A) radiation (370 nm) for 30 minutes. The resultant reaction is similar to photopolymerisation of polymers and is intended to increase the rigidity of the cornea.

Since the development of the Dresden protocol, investigators have proposed several modifications to this original technique. These modifications aim to increase the number of eligible patients that can undergo the CXL procedure in terms of: A) anatomical limitations of patient eligibility (minimum corneal thickness >400µm at the thinnest point) and B) time taken to carry out the procedure (efficiency), while maintaining safety and efficacy.

The Bunsen-Roscoe law of reciprocity states that as long as the irradiance (total energy received by a surface per unit area) remains constant, the photochemical effect should be similar. Thus, theoretically, increasing the intensity of UV-A exposure and reducing treatment time to maintain the same irradiance should yield similar results. The Dresden protocol calls for irradiation with UV-A of 3 mW/cm² for 30 minutes, with a total irradiance of 5.4 J/cm². Modified protocols of accelerated CXL (A-CXL) with the same irradiance as the Dresden protocol have been investigated using UV-A intensities of 6 mW/cm² for 15 minutes, 9 mW/cm² for 10 minutes, 18 mW/cm² for 5 minutes and 30 mW/cm² for 3 minutes.

The exact mechanism of action of CXL is not fully understood, however, it is believed that on a molecular level, CXL is the result of types I and II photodynamic reactions induced by absorption of UV-A by riboflavin which is excited into a triplet state, creating reactive oxygen species which induce covalent bonds within the corneal collagen fibrils, consistent with a type II photochemical reaction, thus increasing the biomechanical strength of the cornea.
Interestingly, CXL performed on ex vivo porcine corneas in a low oxygen environment revealed almost no increase in biomechanical stability, indicating that oxygen is fundamental for cross-linking to occur and is likely the rate limiting substance in the process. Therefore, it is possible that A-CXL modalities may be less effective as they deplete dissolved oxygen in the cornea much more rapidly thus limiting the amount of cross-linking that occurs (at 100µm corneal depth, 30mW/cm\(^2\) reduces oxygen concentration to zero in 1s whereas 3 mW/cm\(^2\) takes 15s to achieve the same effect). However, oxygen concentration rapidly increases once the UV-A exposure is stopped. Once oxygen is depleted the photodynamic reaction switches from type II to the less efficient type I. It is therefore plausible that pulsing (flashing) UV-A of a high intensity may lead to a greater amount of crosslinking due to oxygen being replenished during the ‘off’ period, restarting the type II photodynamic reaction, thus producing more cross-links, resulting in a greater effect on biomechanical properties.

Additionally, the dosage limits to prevent actinic damage to the cornea are 23 J/cm\(^2\) at 360 nm, 27 J/cm\(^2\) at 365 nm and 32 J/cm\(^2\) at 370 nm. Thus, if endothelial damage can be prevented, theoretically a higher total energy of UV-A could be applied in an A-CXL procedure. A-CXL conducted with a higher intensity, has been shown to be safe but not as effective as the Dresden protocol. As a result, recent attempts to improve the efficacy of A-CXL utilise combinations of: a) high intensity UV-A (up to 30 mW/cm\(^2\)) b) higher total energy (7.2 J/cm\(^2\)) c) pulsed UV-A radiation.

**Aims**

1) To determine if high intensity, high energy accelerated continuous and pulsed ultraviolet-A corneal collagen cross-linking is safe for the treatment of progressive keratoconus

2) To determine if high intensity, high energy accelerated continuous and pulsed ultraviolet-A corneal collagen cross-linking is efficacious the treatment of progressive keratoconus

3) To determine if there is a difference in the efficacy between continuous and pulsed, high intensity high energy accelerated ultraviolet-A corneal collagen cross-linking in the treatment of progressive keratoconus

**9.2 Methods**

**9.2.1 Study Protocol and Design**

This study was designed as a randomised, prospective, longitudinal, comparative investigation of two accelerated corneal collagen cross-linking modalities. Participants with eligible eyes were randomly allocated to receive high intensity (30 mW/cm\(^2\)), high energy (7.2 J/cm\(^2\)) corneal collagen-crosslinking with epithelial debridement and either continuous (constant) (CA-CXL) exposure to UV-A or pulsed
(flashed) (PA-CXL) exposure to UV-A. The randomisation of the interventions was established using a random number generator prior to participant recruitment.

Each participant was examined pre-operatively and 1, 3, 6 and 12-months post-operatively. Each examination included assessment of: uncorrected vision, refractive error, best spectacle corrected visual acuity, corneal power and thickness (Pentacam HR), anterior segment optical coherence tomography (AS-OCT) (Heidelberg Spectralis), corneal microstructure (HRTII and Confoscan 4) and corneal biomechanics (CorVis ST). All outcome measures stated below were obtained as per the methodology outlined in Chapter 3, which includes details regarding IVCM and AS-OCT image capture and analysis. All research procedures were carried out within the University of Auckland clinical research laboratory located within the Ophthalmology Department at Greenlane Clinical Centre, ADHB, Auckland, New Zealand. Treatments were performed between July 2014 and December 2015.

9.2.2 Outcome Measures

Clinical Outcomes
- Post-operative complications
  - Corneal scarring
  - Infection
  - Sterile keratitis
- Treatment failure
  - Disease progression

Computerised Corneal Tomography
- Steep simulated keratometry ($K_{\text{steep}}$) (D)
- Flat simulated keratometry ($K_{\text{flat}}$) (D)
- Maximum Corneal Power ($K_{\text{max}}$) (D)
- Central corneal thickness (CCT) (µm)
- Thinnest corneal thickness (TCT) (µm)

Anterior Segment Optical Coherence Tomography
- Depth of post-operative stromal demarcation line (% of central corneal thickness)

Refractive Error and Vision
- Uncorrected vision (UCVA) (LogMAR)
- Best spectacle corrected VA (BSCVA) (LogMAR)
- Spherical equivalent refraction (SE) (D)
In vivo Confocal Microscopy
- Corneal basal epithelial density (cells/mm²)
- Corneal sub-basal nerve density (mm/mm²)
- Anterior keratocyte density (cells/mm²)
- Posterior keratocyte density (cells/mm²)
- Corneal endothelial density (cells/mm²)

Corneal Biomechanics
- Deformation amplitude (DA) (mm)

9.2.3 Participants
Participants were recruited from cornea and anterior segment sub-specialist clinics at the Ophthalmology service within GLCC, ADHB. Potential participants were referred internally to a specialist keratoconus and CXL treatment clinic, from which eligible participants were invited to participate in the study. If both eyes of a participant were eligible during the study enrolment period, the participant was offered the option of enrolling both eyes. Each eye was considered as separate but was allocated the other type of UV-A to negate any subject specific factors.

This investigation adhered to the tenets of the Declaration of Helsinki and ethical approval for the study was obtained from the Northern X Ethics division of the Health and Disability Ethics Committee (HDEC) of NZ, approval reference: NTX/08/08/070AM02.

Inclusion Criteria
- Documented progressive keratoconus
- Age >14 years at enrolment
- Thinnest corneal thickness >400 μm
- No corneal scarring on slit-lamp biomicroscopy
- The ability to understand the implications of the study, such that the participant was able to provide fully informed written consent or a parent/guardian was able to do so if the participant was less than 16 years of age.
Exclusion Criteria

- Previous/current episode of corneal hydrops in either eye
- Previous ocular surgery or trauma
- Systemic disease which may affect the cornea (e.g. diabetes mellitus)
- Corneal tomographically stable keratoconus in the preceding 12-months
- Previous corneal herpetic disease
- Thinnest corneal thickness <400 μm
- Corneal stromal scarring on slit-lamp biomicroscopy
- Any other ocular pathology
- Inability to give informed consent
- Inability to attend follow-up appointments over the study period

Definition of Keratoconus Progression

- At least 6 months of preceding visual/refractive/topographic/keratometric data available to accurately assess the rate of progression. Progression of keratoconus 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
  - Decrease in corneal thickness of ≥30μm in the preceding 6 months
  - Loss of 2 or more lines of best spectacle corrected visual acuity

Statistical Analysis

The Kolmogorov-Smirnov test was performed to confirm that all study parameters were parametric. The independent samples t-test was used to analyse the difference in the outcome measures between the eyes in the CA-CXL and PA-CXL treatment groups at baseline. The paired samples t-test was used to analyse intra-group differences at different post-operative time points relative to baseline to determine progression. The independent samples t-test was utilised to assess differences in the treatment groups at different post-operative time points relative to the baseline visit. The chi square test was used to assess differences in the proportions of participants in the CA-CXL and PA-CXL treatment groups that experienced clinical complications, treatment failure and developed a corneal stromal demarcation line post-operatively.

All analyses were performed using SPSS 22.0 for Windows (Chicago, IL, USA). A p-value of <0.05 was considered as significant throughout.
9.2.4 Surgical Procedure of Corneal Collagen Cross-linking

The Avedro KXL (A-KXL) (Avedro Inc., Waltham, MS, USA) (Figure 9-1) is an advanced ultraviolet-A (UV-A) emitting device, designed specifically to carry out corneal collagen cross-linking in various capacities, including treatment for progressive keratoconus. The device consists primarily of a battery, central processing unit and touch display interface, positioning arm and UV-A light source (365 nm) (Figure 9-1A.). The A-KXL contains a highly programmable, semi-automated interface allowing for customisable control of four factors:

1. Riboflavin induction period (duration of riboflavin application, ranging from 0-30 minutes)
2. Continuous (constant) or pulsed (flashing rate of 1 second on/1 second off (0.5 Hz)) UV-A
3. Total energy (ranging from 1.0-10.7 J/cm²) (safety limitation set at 7.2 J/cm²)
4. Intensity of UV-A (ranging from 15-45 mW/cm²)

The UV-A source also contains a targeting system, consisting of red cross-hairs and a green dot, which are projected onto the participant’s cornea. Movement of the UV-A source in the x and y-axis moves both components of the targeting system in the same direction as the UV-A source, while movement in the z-axis results in stationary red-cross hairs but movement of the green dot relative to the cross-hairs. Once the cross-hairs are aligned by manually moving the UV-A source into place above the central cornea (x and y-axis alignment), the UV-A source is moved up and down until the position of the green dot coincides with the centre of the cross-hairs (8mm above the cornea).*

Once a treatment is programmed and initiated, the A-KXL goes through two phases: the riboflavin induction period and the UV-A treatment period. Both phases are semi-automated as each must be manually initiated by the operator.

The operator initiates the riboflavin induction period and begins applying the topical riboflavin. The display indicates how long riboflavin has been applied and how much longer it should be applied by means of count-up and count-down timers respectively. Once the riboflavin induction period ends, the operator aligns the UV-A source and initiates the UV-A treatment period, the UV-A source begins to emit UV-A as programmed.

The UV-A treatment period has analogous timers to the riboflavin induction period as well as indicators of the amount of UV-A applied (in Joules) and the amount still to be applied. The UV-A treatment can be paused and continued at will, as alignment may need to be adjusted during the

* The green dot did not photograph well but alignment of the red cross-hairs is visible in Figure 9-2E.
application of the UV-A. Three specific auditory notifications are provided every two minutes during the phase, 10 seconds prior to completion and at completion, respectively, during both the riboflavin induction period and UV-A treatment periods.

Figure 9-1 The Avedro KXL (A-KXL) (Avedro Inc., Waltham, MS, USA) A. The A-KXL, arrows indicate the battery housing, central processing unit (CPU) and touch display, positioning arm and ultraviolet-A (UV-A) light source B. A-KXL settings for the continuous treatment group C. A-KXL settings for the pulsed treatment group
The treatment protocols utilised in this study were carried out to the specifications set out by the manufacturer of the A-KXL (Avedro Inc., Waltham, MS, USA) for high intensity, CA-CXL and PA-CXL for progressive keratoconus. This protocol was adapted from the original reported by Wollensak et al.,\textsuperscript{13} taking into account the actinic dose exposure limits of the cornea\textsuperscript{305} and the proprietary formula of the riboflavin produced by Avedro (VibeX Rapid). The procedures were carried out under aseptic conditions in a dedicated procedure room. The procedure itself is detailed below:

**Epithelial Debridement**

I. The participant was asked to lie in the supine position and the treatment eye confirmed

II. Preservative free tetracaine hydrochloride 1% eye drops (Chauvin Pharmaceuticals, Surrey, UK) were instilled onto the eye and applied every 4-5 minutes throughout the procedure

III. The external eye and periocular structures was disinfected with 10% povidone iodine solution (Pfizer Australia Pty Ltd, N.S.W., Australia) for 1 minute then cleaned

IV. A sterile drape was placed over the eye and a sterile eyelid speculum inserted

V. Prior to epithelial debridement, central corneal thickness was assessed using the Takagi Handy Pachymeter P-1 (Takagi Seiko Co. Ltd., Takaoka, Japan) (Figure 9-1C.) to ensure adequate central corneal thickness

VI. A Hoffer 8mm optical zone marker with cross-hairs was applied to the cornea and centred, followed by application of 20% ethanol solution into the well of the optical zone marker and kept in contact with the epithelium for 20 to 30 seconds, then absorbed using a dry polyvinyl alcohol (PVA) spear (Eyecare). The eye was rinsed with balanced saline solution (BSS) and the epithelium mechanically debrided using a Tooke’s knife creating a central 9mm diameter circular epithelial defect

VII. The thickness of the remaining central corneal bed was determined with pachymetry

**Riboflavin Application**

VIII. The riboflavin induction period was initiated on the A-KXL and riboflavin drops were instilled every 5-10 seconds for 10 minutes to completely cover and saturate the corneal stroma. The riboflavin solution used in both treatment groups was VibeX Rapid (Avedro Inc., Waltham, MS, USA) composed of dextran-free riboflavin 0.1% with hypromellose (hydroxypropyl methylcellulose)

IX. Central corneal pachymetry was performed following the 10 minute soak with riboflavin solution to ensure that CCT remained thicker than 400 μm. If the CCT fell below 400 μm, sterile water was instilled until the CCT was greater than 400 μm
UV-A Irradiation

X. The UV-A source was aligned and UV-A treatment commenced on the A-KXL. The cornea was wetted with BSS as needed to prevent drying during the UV-A treatment

XI. Irradiation was performed for 4 minutes in the continuous group (continuous irradiation) and 8 minutes in the pulsed group (pulsed irradiation; 1 second on, 1 second off) at 30 mW/cm² – 7.2 J/cm² total energy in both cases

Post-operative Care

XII. Following irradiation the eye was flushed with BSS, a bandage contact lens inserted (Bioinfinity, CooperVision, Fairport, NY) and a drop of preservative free cyclopentolate 1% and chloramphenicol 0.5% eye drops (Chauvin Pharmaceuticals, Surrey, UK) instilled

XIII. The participant was provided with a prescription for chloramphenicol 0.5% and fluoromethalone 0.1% (Flucon) eye drops (Alcon, Fort Worth, TX, USA) and systemic pain relief, oral Tramadol (50mg every 4-6 hours). 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. Each participant was provided with a detailed instruction sheet regarding the topical antibiotic, corticosteroid and follow-up appointment regimen

XIV. Post-operative clinical examinations were scheduled for day 1, day 3, day 7, 1 month, 3 months, 6 months and 12-months

XV. The bandage contact lens was removed at day 3 or 7, depending on when complete re-epithelialisation occurred
Figure 9-2 Surgical Procedure of Corneal Collagen Cross-linking

A. 8mm optical zone marker with cross-hairs, Tooke’s knife and PVA spear
B. Epithelial debridement using the Tooke’s knife
C. Takagi Handy Pachymeter P-1 (Takagi Seiko Co. Ltd., Takaoka, Japan)
D. Application of riboflavin solution (VibeX Rapid)
E. Alignment of red cross-hairs and green alignment dot prior to initialising ultraviolet-A treatment
F. Active ultraviolet-A treatment – the appearance of the pulsed treatment is essentially oscillation between E. and F. in 1 second intervals
9.3 Results

CXL was performed on 57 eyes of 50 participants. The CA-CXL group consisted of 29 eyes of 29 participants, while the PA-CXL group consisted of 28 eyes of 28 participants. The mean age of the CA-CXL group was 20.6 ± 5.4 years, with 53% male participants, while the mean age of the PA-CXL group was 21.6 ± 6.8 years, 56% male. There were no differences in age (p=0.641) and gender distribution (p=0.508) between the groups.

9.3.1 Clinical complications

The corneal epithelial defect had completely healed at day 3 in all but 2 (3.5%) eyes; however, there was complete re-epithelialisation at day 7 in both remaining eyes.

There were no (0%) cases of microbial keratitis or sterile corneal infiltrates in the study cohort. Anterior stromal haze was noted in 46 (81%) eyes at the 1 month follow-up (Figure 9-3), however this resolved by 3-months in all but 4 (7%) eyes of 4 participants, 3 from the continuous group and 1 from the pulsed group (p=0.319), in which case the haze persisted as a localised stromal opacification even at 12-months.

Figure 9-3 Post-accelerated corneal collagen cross-linking anterior corneal stromal haze observed 1-month postoperatively through a slit-lamp biomicroscope A. 16x magnification B. 25x magnification
9.3.2 Vision and Refraction

9.3.2.1 Vision

The mean UCVA and BCVA at each examination pre and post-operatively for both the CA-CXL and PA-CXL groups is detailed in Table 9-1. The trend in these parameters at the same time points is illustrated in Figure 9-4. There was no difference in UCVA and BSCVA at baseline between the CA-CXL and PA-CXL groups (p>0.25 in all cases). The CA-CXL group demonstrated a slight improvement in UCVA relative to pre-operatively at 3-months (-0.11 ± 0.27 LogMAR) which persisted at 12-months where it appears to have improved further (-0.18 ± 0.36 LogMAR). UCVA improved relative to pre-operatively in the PA-CXL group at 6-months (-0.12 ± 0.14 LogMAR), however, the improvement at 12-months relative to baseline was not significant. Only eyes in the CA-CXL group showed significant improvement in BSCVA, apparent at 3, 6 and 12-months.

Table 9-1 Uncorrected Vision (UCVA) and Best Spectacle Corrected Visual Acuity (BSCVA) pre-operatively at 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation. The p-values in the pre-operative row of the Pulsed group represent the result of independent sample t-test between continuous and pulsed groups at the pre-operative stage. All subsequent p-values pertain to the paired-samples t-test for that variable compared to the pre-operative state. All parameter values are mean ± standard deviation.
Figure 9-4 Mean Uncorrected Vision (UCVA) and Best Spectacle Corrected Visual Acuity (BSCVA) preoperatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation

Table 9-2 contains the analysis of the change in UCVA and BCVA between the pre-operative state and 1, 3, 6 and 12-month post-operative in the CA-CXL and PA-CXL groups. The CA-CXL group demonstrated significant improvement in UCVA and BSCVA at 3 and 12 and 3, 6 and 12 months relative to baseline, yet there was no difference in the improvement in UCVA and BCVA between the CA-CXL and PA-CXL groups, despite the PA-CXL group only showing significant improvement in UCVA at 6-months (p>0.1 in all cases).

Table 9-2 Comparison of change in Uncorrected Vision (UCVA) and Best Spectacle Corrected Visual Acuity (BSCVA) pre-operatively and 1, 3, 6 and 12-months post-operatively. All parameter values are the mean difference ± standard deviation of the variable compared to the pre-operative state. P-values pertain to the comparison of the change observed in the continuous and pulsed irradiation groups at the specified time point

<table>
<thead>
<tr>
<th>Examination Time Point (months)</th>
<th>UCVA</th>
<th>BSCVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Continuous</td>
<td>Pulsed</td>
</tr>
<tr>
<td>1-month</td>
<td>26</td>
<td>0.01 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>p=0.805</td>
<td></td>
</tr>
<tr>
<td>3-months</td>
<td>25</td>
<td>-0.11 ± 0.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.652</td>
</tr>
<tr>
<td>6-months</td>
<td>26</td>
<td>-0.06 ± 0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.323</td>
</tr>
<tr>
<td>12-months</td>
<td>20</td>
<td>-0.18 ± 0.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.568</td>
</tr>
</tbody>
</table>
9.3.2.2 Refraction

A significant reduction in the mean spherical equivalent refraction was noted at 3, 6 and 12-months post-operatively, compared to pre-operatively, in the CA-CXL group, while there was only a significant reduction at 12-months in the PA-CXL group (p<0.04 in all cases) (Table 9-3 and Figure 9-5). There was no difference in the mean change in spherical equivalent refraction at 1, 3, 6 and 12-months post-operatively between the CA-CXL and PA-CXL groups (Table 9-4).

Table 9-3 Spherical equivalent refraction pre-operatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation. The p-values in the pre-operative row of the pulsed group represent the result of independent sample t-test between continuous and pulsed groups at the pre-operative stage. All subsequent p-values pertain to the paired-samples t-test for that variable compared to the pre-operative state. All parameter values are mean ± standard deviation

<table>
<thead>
<tr>
<th>Examination</th>
<th>N</th>
<th>Continuous</th>
<th>P-Value</th>
<th>N</th>
<th>Pulsed</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Op</td>
<td>29</td>
<td>-3.18 ± 4.39</td>
<td>NA</td>
<td>28</td>
<td>-2.95 ± 4.91</td>
<td>0.205</td>
</tr>
<tr>
<td>1-month</td>
<td>26</td>
<td>-2.94 ± 4.55</td>
<td>0.147</td>
<td>24</td>
<td>-1.57 ± 2.61</td>
<td>0.135</td>
</tr>
<tr>
<td>3-months</td>
<td>25</td>
<td>-2.50 ± 4.23</td>
<td>0.010</td>
<td>21</td>
<td>-2.38 ± 4.10</td>
<td>0.753</td>
</tr>
<tr>
<td>6-months</td>
<td>26</td>
<td>-1.39 ± 3.62</td>
<td>0.001</td>
<td>23</td>
<td>-1.86 ± 4.34</td>
<td>0.598</td>
</tr>
<tr>
<td>12-months</td>
<td>20</td>
<td>-1.50 ± 3.59</td>
<td>&lt;0.001</td>
<td>19</td>
<td>-1.51 ± 2.75</td>
<td>0.036</td>
</tr>
</tbody>
</table>

Table 9-4 Comparison of mean change in spherical equivalent refraction pre-operatively and 1, 3, 6 and 12-months post-operatively. All parameter values are the mean difference ± standard deviation of the variable compared to the pre-operative state. P-values pertain to the comparison of the change observed in the continuous and pulsed irradiation groups at the specified time point

<table>
<thead>
<tr>
<th>Examination</th>
<th>N</th>
<th>Continuous</th>
<th>P-Value</th>
<th>N</th>
<th>Pulsed</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-month</td>
<td>26</td>
<td>0.51 ± 1.68</td>
<td></td>
<td>24</td>
<td>1.74 ± 1.47</td>
<td>0.301</td>
</tr>
<tr>
<td>3-months</td>
<td>25</td>
<td>1.00 ± 1.82</td>
<td></td>
<td>21</td>
<td>0.56 ± 1.39</td>
<td>0.387</td>
</tr>
<tr>
<td>6-months</td>
<td>26</td>
<td>1.41 ± 1.86</td>
<td></td>
<td>23</td>
<td>1.13 ± 3.30</td>
<td>0.156</td>
</tr>
<tr>
<td>12-months</td>
<td>20</td>
<td>2.06 ± 2.05</td>
<td></td>
<td>19</td>
<td>1.63 ± 2.91</td>
<td>0.348</td>
</tr>
</tbody>
</table>
9.3.3 Computerised Corneal Tomography

9.3.3.1 Anterior Power/Curvature

Results for the analyses concerning the anterior corneal power/curvature in the CA-CXL and PA-CXL groups pre-operatively and at the 1, 3, 6 and 12 month post-operative stages is shown in Tables 9-6 and 9-7 and Figure 9-6.

The mean $K_{\text{Flat}}$ and $K_{\text{Steep}}$ increased significantly 1 month post-operatively, compared to baseline, in both the CA-CXL and PA-CXL groups ($p<0.025$ in both cases). There was no difference in the mean $K_{\text{Flat}}$, $K_{\text{Steep}}$, and $K_{\text{Max}}$ pre-operatively between the CA-CXL and PA-CXL groups. There was no difference in the mean $K_{\text{Flat}}$, $K_{\text{Steep}}$, and $K_{\text{Max}}$ at 3, 6 and 12-months post-operatively, compared to baseline in both the CA-CXL and PA-CXL groups ($p>0.12$ in all cases) (Table 9-5). Furthermore, there was no difference in the mean change in $K_{\text{Flat}}$, $K_{\text{Steep}}$ and $K_{\text{Max}}$ at 1, 3, 6 and 12-months post-operatively between the CA-CXL and PA-CXL groups ($p>0.55$ in all cases) (Table 9-6).
Table 9-5 Anterior corneal power/curvature (D), K_{Flat}, K_{Steep} and K_{Max}, pre-operatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation. The p-values in the pre-operative row of the Pulsed group represent the result of independent sample t-test between continuous and pulsed groups at the pre-operative stage. All subsequent p-values pertain to the paired-samples t-test for that variable compared to the pre-operative state. All parameter values are mean ± standard deviation.

<table>
<thead>
<tr>
<th>Examination</th>
<th>Continuous</th>
<th>Pulsed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>K_{Flat}</td>
</tr>
<tr>
<td>Pre-Op</td>
<td>29</td>
<td>45.8 ± 3.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.340</td>
</tr>
<tr>
<td>1-month</td>
<td>26</td>
<td>46.1 ± 3.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.017</td>
</tr>
<tr>
<td>3-months</td>
<td>25</td>
<td>45.8 ± 3.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.187</td>
</tr>
<tr>
<td>6-months</td>
<td>26</td>
<td>45.7 ± 3.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.733</td>
</tr>
<tr>
<td>12-months</td>
<td>20</td>
<td>45.5 ± 3.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.731</td>
</tr>
</tbody>
</table>

Table 9-6 Comparison of change in anterior corneal power/curvature, K_{Flat}, K_{Steep} and K_{Max}, pre-operatively and 1, 3, 6 and 12-months post-operatively. All parameter values are the mean difference ± standard deviation of the variable compared to the pre-operative state. P-values pertain to the comparison of the change observed in the continuous and pulsed irradiation groups at the specified time point.

<table>
<thead>
<tr>
<th>Examination</th>
<th>Continuous</th>
<th>Pulsed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>K_{Flat}</td>
</tr>
<tr>
<td>1-month</td>
<td>26</td>
<td>0.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.555</td>
</tr>
<tr>
<td>3-months</td>
<td>25</td>
<td>0.2 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.743</td>
</tr>
<tr>
<td>6-months</td>
<td>26</td>
<td>0.1 ± 1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.683</td>
</tr>
<tr>
<td>12-months</td>
<td>20</td>
<td>-0.1 ± 1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.747</td>
</tr>
</tbody>
</table>
Figure 9-6 Mean anterior corneal power/curvature, $K_{\text{Flat}}$, $K_{\text{Steep}}$ and $K_{\text{Max}}$, pre-operatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation

9.3.3.2 Corneal Thickness

Table 9-7 and Figure 9-7 demonstrate the mean corneal thickness, CCT and TCT, pre-operatively and 1, 3, 6 and 12 month post-operatively. There was no difference in the mean CCT and TCT pre-operatively between the CA-CXL and PA-CXL groups, additionally, there was no difference in the mean CCT and TCT at 1, 3, 6 and 12-months post-operatively, compared to baseline, in both the CA-CXL and PA-CXL groups ($p > 1.3$ in all cases).

Furthermore, there was no difference in the mean change in CCT and TCT at 1, 3, 6 and 12-months post-operatively, compared to baseline, in both the CA-CXL and PA-CXL groups ($p > 0.05$ in all cases) (Table 9-8).
Table 9-7 Corneal thickness, central corneal thickness (CCT) and thinnest corneal thickness (TCT), in eyes treated with continuous and pulsed ultraviolet-A irradiation. The p-values in the pre-operative row of the Pulsed group represent the result of independent sample t-test between continuous and pulsed groups at the pre-operative stage. All subsequent p-values pertain to the paired-samples t-test for that variable compared to the pre-operative state. All parameter values are mean ± standard deviation

<table>
<thead>
<tr>
<th>Examination</th>
<th>Continuous</th>
<th>Pulsed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CCT</td>
<td>TCT</td>
</tr>
<tr>
<td>Pre-Op</td>
<td>489.8 ± 28.5</td>
<td>458.9 ± 37.6</td>
</tr>
<tr>
<td></td>
<td>p=0.777</td>
<td></td>
</tr>
<tr>
<td>1-month</td>
<td>487.2 ± 35.5</td>
<td>465.2 ± 35.7</td>
</tr>
<tr>
<td></td>
<td>p=0.491</td>
<td></td>
</tr>
<tr>
<td>3-months</td>
<td>481.8 ± 31.5</td>
<td>456.9 ± 31.3</td>
</tr>
<tr>
<td></td>
<td>p=0.210</td>
<td></td>
</tr>
<tr>
<td>6-months</td>
<td>482.0 ± 27.2</td>
<td>460.2 ± 28.2</td>
</tr>
<tr>
<td></td>
<td>p=0.286</td>
<td></td>
</tr>
<tr>
<td>12-months</td>
<td>476.2 ± 29.5</td>
<td>452.2 ± 27.3</td>
</tr>
<tr>
<td></td>
<td>p=0.331</td>
<td></td>
</tr>
</tbody>
</table>

Table 9-8 Comparison of change corneal thickness, CCT and TCT, pre-operatively and 1, 3, 6 and 12-months post-operatively. All parameter values are the mean difference ± standard deviation of the variable compared to the pre-operative state. P-values pertain to the comparison of the change observed in the continuous and pulsed irradiation groups at the specified time point

<table>
<thead>
<tr>
<th>Examination</th>
<th>Continuous</th>
<th>Pulsed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CCT</td>
<td>TCT</td>
</tr>
<tr>
<td>1-month</td>
<td>-2.6 ± 18.4</td>
<td>6.3 ± 40.2</td>
</tr>
<tr>
<td></td>
<td>p=0.513</td>
<td></td>
</tr>
<tr>
<td>3-months</td>
<td>-3.3 ± 12.5</td>
<td>-4.6 ± 14.6</td>
</tr>
<tr>
<td></td>
<td>p=0.056</td>
<td></td>
</tr>
<tr>
<td>6-months</td>
<td>-2.8 ± 12.4</td>
<td>-0.2 ± 12.9</td>
</tr>
<tr>
<td></td>
<td>p=0.631</td>
<td></td>
</tr>
<tr>
<td>12-months</td>
<td>-3.7 ± 16.7</td>
<td>-5.8 ± 16.8</td>
</tr>
<tr>
<td></td>
<td>p=0.710</td>
<td></td>
</tr>
</tbody>
</table>
Figure 9-7 Mean corneal thickness, CCT and TCT, pre-operatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation

9.3.4 Anterior Segment Optical Coherence Tomography

9.3.4.1 Depth of Demarcation

The proportion of eyes that developed a post-operative stromal demarcation line and the percentage of corneal thickness at which the demarcation line was located are presented in Table 9-9. Compared to the CA-CXL treatment group at 1, 3, 6 and 12-months post-operatively, a similar proportion of participants developed a stromal demarcation line in the PA-CXL treatment group and there was no difference in the mean percentage of thickness depth between the groups (p>0.09 in all cases).

Table 9-9 Depth of demarcation (% corneal thickness) in eyes treated with continuous and pulsed ultraviolet-A irradiation. N indicates the number of participants assessed that had a demarcation line. P-value1 indicates the result of the independent samples t-test comparing the depth of demarcation in the continuous and pulsed groups at different post-operative time points, while P-Value2 indicates the result of the chi square test analysing the difference in the proportion of participants that had a demarcation line in the continuous and pulsed groups.
Figure 9-8 Demarcation line (indicated by the arrows) on anterior segment optical coherence tomography A. Shallow demarcation line B. Deep demarcation line

9.3.5 In vivo Confocal Microscopy

9.3.5.1 Corneal Basal Epithelium

Following epithelial debridement during the procedure, the mean corneal basal epithelial cell density was at baseline levels 1-month post-operatively and persisted at these levels at 3, 6 and 12-months post-operatively in both the continuous and PA-CXL groups (Table 9-10).

Table 9-10 Corneal basal epithelial cell density pre-operatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation. P-values pertain to the paired-samples t-test for that variable compared to the pre-operative state. All parameter values are mean ± standard deviation

<table>
<thead>
<tr>
<th>Examination</th>
<th>N</th>
<th>Continuous</th>
<th>P-Value</th>
<th>N</th>
<th>Pulsed</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Op</td>
<td>29</td>
<td>4613 ± 743</td>
<td>NA</td>
<td>28</td>
<td>4357 ± 796</td>
<td>NA</td>
</tr>
<tr>
<td>1-month</td>
<td>26</td>
<td>4549 ± 820</td>
<td>0.427</td>
<td>24</td>
<td>4308 ± 633</td>
<td>0.724</td>
</tr>
<tr>
<td>3-months</td>
<td>25</td>
<td>4640 ± 775</td>
<td>0.669</td>
<td>21</td>
<td>4604 ± 9.31</td>
<td>0.538</td>
</tr>
<tr>
<td>6-months</td>
<td>26</td>
<td>4564 ± 574</td>
<td>0.304</td>
<td>23</td>
<td>4552 ± 667</td>
<td>0.495</td>
</tr>
<tr>
<td>12-months</td>
<td>20</td>
<td>4353 ± 409</td>
<td>0.163</td>
<td>19</td>
<td>4739 ± 705</td>
<td>0.584</td>
</tr>
</tbody>
</table>
9.3.5.2 Corneal Sub-basal Nerve Plexus

The results of the analyses of the corneal sub-basal nerve plexus density pre-operatively and 1, 3, 6 and 12-months post-operatively was similar in the CA-CXL and PA-CXL groups, with a significant reduction in mean density at 1, 3 and 6 months post-operatively, recovering to baseline levels 12-months post-operatively (Table 9-11 and Figure 9-9).

Table 9-11 Corneal sub-basal nerve plexus density pre-operatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation. P-values pertain to the paired-samples t-test for that variable compared to the pre-operative state. All parameter values are mean ± standard deviation

<table>
<thead>
<tr>
<th>Examination</th>
<th>Continuous</th>
<th>P-Value</th>
<th>N</th>
<th>Pulsed</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Op</td>
<td>29</td>
<td>18.1 ± 5.3</td>
<td>NA</td>
<td>28</td>
<td>14.8 ± 5.2</td>
</tr>
<tr>
<td>1-month</td>
<td>26</td>
<td>0.7 ± 2.9</td>
<td>&lt;0.001</td>
<td>24</td>
<td>0.2 ± 0.5</td>
</tr>
<tr>
<td>3-months</td>
<td>25</td>
<td>2.2 ± 4.4</td>
<td>&lt;0.001</td>
<td>21</td>
<td>2.4 ± 4.1</td>
</tr>
<tr>
<td>6-months</td>
<td>26</td>
<td>8.6 ± 6.2</td>
<td>&lt;0.001</td>
<td>23</td>
<td>8.3 ± 5.5</td>
</tr>
<tr>
<td>12-months</td>
<td>20</td>
<td>16.3 ± 4.5</td>
<td>0.440</td>
<td>19</td>
<td>14.3 ± 6.2</td>
</tr>
</tbody>
</table>

Figure 9-9 Corneal sub-basal nerve plexus A. Pre-operative B. Reduced density 1-month post-operatively C. Return to pre-operative density 12-months post-operatively
9.3.5.3 Keratocytes

Similar to the sub-basal corneal nerve plexus density, the results of the analyses of the keratocyte density pre-operatively and 1, 3, 6 and 12-months post-operatively was similar in the CA-CXL and PA-CXL groups.

There was a significant reduction in mean anterior keratocyte density 1, 3, 6 and 12-months post-operatively, in both groups. However, there was no difference in the mean posterior keratocyte density 1, 3, 6 and 12-months post-operatively, compared to baseline in both groups (Table 9-12 and Figure 9-10).

Table 9-12 Anterior and posterior keratocyte density pre-operatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation. P-values pertain to the paired-samples t-test for that variable compared to the pre-operative state. All parameter values are mean ± standard deviation

<table>
<thead>
<tr>
<th>Examination</th>
<th>N</th>
<th>Anterior Density (cells/mm²)</th>
<th>Posterior Density (cells/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Op</td>
<td>29</td>
<td>544 ± 103</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>281 ± 53</td>
<td></td>
</tr>
<tr>
<td>1-month</td>
<td>26</td>
<td>126 ± 163</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>295 ± 80 p=0.344</td>
<td></td>
</tr>
<tr>
<td>3-months</td>
<td>25</td>
<td>325 ± 283</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>280 ± 54 p=0.698</td>
<td></td>
</tr>
<tr>
<td>6-months</td>
<td>26</td>
<td>405 ± 217</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.028</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>281 ± 63 p=0.500</td>
<td></td>
</tr>
<tr>
<td>12-months</td>
<td>20</td>
<td>435 ± 156</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.043</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>283 ± 57 p=0.531</td>
<td></td>
</tr>
<tr>
<td>Pulsed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Op</td>
<td>28</td>
<td>637 ± 168</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>283 ± 42</td>
<td></td>
</tr>
<tr>
<td>1-month</td>
<td>24</td>
<td>75 ± 134</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>280 ± 41 p=0.648</td>
<td></td>
</tr>
<tr>
<td>3-months</td>
<td>21</td>
<td>319 ± 239</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>381 ± 49 p=0.623</td>
<td></td>
</tr>
<tr>
<td>6-months</td>
<td>23</td>
<td>403 ± 196</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>285 ± 36 p=0.723</td>
<td></td>
</tr>
<tr>
<td>12-months</td>
<td>19</td>
<td>439 ± 106</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>278 ± 51 p=0.277</td>
<td></td>
</tr>
</tbody>
</table>

Figure 9-10 Anterior keratocytes A. Pre-operative B. Hyper-reflective cytoplasm and extracellular lacunae 1-month post-operatively C. Persisting reduction in density 12-months post-operatively
9.3.5.4 Corneal Endothelium
There was no difference in the mean corneal endothelial cell density post-operatively and 1, 3, 6 and 12-months post-operatively compared to the pre-operative state in both the CA-CXL and PA-CXL groups (Table 9-13).

Table 9-13 Corneal endothelial cell density pre-operatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation. P-values pertain to the paired-samples t-test for that variable compared to the pre-operative state. All parameter values are mean ± standard deviation

<table>
<thead>
<tr>
<th>Examination</th>
<th>Continuous</th>
<th>P-Value</th>
<th>N</th>
<th>Pulsed</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Op</td>
<td>2608 ± 377</td>
<td>NA</td>
<td>29</td>
<td>2541 ± 402</td>
<td>NA</td>
</tr>
<tr>
<td>1-month</td>
<td>2537 ± 511</td>
<td>0.186</td>
<td>26</td>
<td>2572 ± 414</td>
<td>0.102</td>
</tr>
<tr>
<td>3-months</td>
<td>2542 ± 497</td>
<td>0.108</td>
<td>25</td>
<td>2618 ± 344</td>
<td>0.265</td>
</tr>
<tr>
<td>6-months</td>
<td>2535 ± 524</td>
<td>0.236</td>
<td>26</td>
<td>2564 ± 318</td>
<td>0.399</td>
</tr>
<tr>
<td>12-months</td>
<td>2480 ± 504</td>
<td>0.115</td>
<td>20</td>
<td>2521 ± 409</td>
<td>0.589</td>
</tr>
</tbody>
</table>

Figure 9-11 Corneal endothelial cells, high density pre-operatively (A.) persisting at 12-months post-operatively (B.)
9.3.6 Corneal Biomechanics

9.3.6.1 Deformation Amplitude

There was no significant difference in the mean DA pre-operatively between the CA-CXL and PA-CXL groups. There was also no significant difference in the mean deformation amplitude at 1, 3, 6 and 12-months post-operatively, compared to baseline, in both treatment groups (p>0.25 in all cases) (Table 9-14 and Figure 9-12). There was no significant difference in the mean change in DA compared to the pre-operative state, 1, 3, 6 and 12-months post-operatively in both the CA-CXL and PA-CXL groups (p>0.13 in all cases) (Table 9-15).

Table 9-14 Deformation amplitude pre-operatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A irradiation. The p-value in the pre-operative row represents the result of independent sample t-test between continuous and pulsed groups at the pre-operative stage. All other p-values pertain to the paired-samples t-test for that variable compared to the pre-operative state. All parameter values are mean ± standard deviation

<table>
<thead>
<tr>
<th>Examination</th>
<th>N</th>
<th>Continuous</th>
<th>P-Value</th>
<th>N</th>
<th>Pulsed</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Op</td>
<td>29</td>
<td>1.198 ± 0.112</td>
<td>NA</td>
<td>28</td>
<td>1.193 ± 0.108</td>
<td>p=0.955</td>
</tr>
<tr>
<td>1-month</td>
<td>26</td>
<td>1.198 ± 0.142</td>
<td>0.563</td>
<td>24</td>
<td>1.176 ± 0.122</td>
<td>0.375</td>
</tr>
<tr>
<td>3-months</td>
<td>25</td>
<td>1.210 ± 0.137</td>
<td>0.490</td>
<td>21</td>
<td>1.229 ± 0.112</td>
<td>0.254</td>
</tr>
<tr>
<td>6-months</td>
<td>26</td>
<td>1.181 ± 0.104</td>
<td>0.389</td>
<td>23</td>
<td>1.209 ± 0.099</td>
<td>0.572</td>
</tr>
<tr>
<td>12-months</td>
<td>20</td>
<td>1.214 ± 0.167</td>
<td>0.932</td>
<td>19</td>
<td>1.230 ± 0.130</td>
<td>0.228</td>
</tr>
</tbody>
</table>

Figure 9-12 Mean deformation amplitude pre-operatively and 1, 3, 6 and 12-months post-operatively in eyes treated with continuous and pulsed ultraviolet-A
Table 9-15 Comparison of change deformation amplitude pre-operatively and 1, 3, 6 and 12-months post-operatively. All parameter values are the mean difference ± standard deviation of the variable compared to the pre-operative state. P-values pertain to the comparison of the change observed in the continuous and pulsed irradiation groups at the specified time point

<table>
<thead>
<tr>
<th>Examination</th>
<th>N</th>
<th>Continuous</th>
<th>N</th>
<th>Pulsed</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-month</td>
<td>26</td>
<td>0.064 ± 0.271</td>
<td>24</td>
<td>-0.020 ± 0.107</td>
<td>0.135</td>
</tr>
<tr>
<td>3-months</td>
<td>25</td>
<td>0.057 ± 0.245</td>
<td>21</td>
<td>0.016 ± 0.072</td>
<td>0.157</td>
</tr>
<tr>
<td>6-months</td>
<td>26</td>
<td>-0.034 ± 0.246</td>
<td>23</td>
<td>0.011 ± 0.095</td>
<td>0.273</td>
</tr>
<tr>
<td>12-months</td>
<td>20</td>
<td>0.068 ± 0.308</td>
<td>19</td>
<td>0.027 ± 0.094</td>
<td>0.218</td>
</tr>
</tbody>
</table>

9.3.7 Treatment Failure

Disease progression was assessed at 12-months post-operatively and showed that just 3 (7.6%) eyes had an increase in maximum keratometry of ≥0.75D at 12-months (2 (10%) CA-CXL, 1 (5%) PA-CXL, p=0.520).

9.4 Discussion

In terms of safety, CXL carries the risk of a number of complications including; infection, sterile or infectious infiltrates, transient corneal haze and scarring, most of these adverse events are associated with removal of the epithelium. Many of these risks are significantly reduced with a regimen of topical antibiotics and corticosteroids post-operatively.

The Dresden protocol has previously been associated with sterile infiltrates in 7.6% of cases, and stromal scarring and loss of 2 lines of vision or more in 2.9%. However, transient post-operative corneal haze is observed in the majority of cases (up to 90%) following the Dresden protocol. In contrast, infection following CXL is a rare complication; a recent large retrospective study revealed an infection rate of 0.002% following the Dresden protocol and no cases of infection were reported following A-CXL.

The current study demonstrates a similar, low rate of stromal scarring (7%) to previously reported investigations of the Dresden protocol. Furthermore, no cases of corneal infection or sterile infiltrates were recorded. Thus in this respect, the risk profile of the high intensity, high irradiance CXL protocol used in this study is similar to that of the well-established Dresden protocol.

Other aspects of the safety of a CXL procedure are related to the effects of the procedure on the corneal microstructure. In vivo confocal microscopy has been used extensively to investigate the

*Unless stated otherwise, all ultraviolet-A irradiances (mW/cm²) outlined in this section are for protocols utilising irradiance of 5.2 J/cm²*
microstructural changes that occur following CXL. Following treatment using the Dresden protocol; the central corneal sub-basal nerve plexus is often completely obliterated and a significant decrease in anterior keratocyte density (due to apoptosis) is observed at 1-6 months post-operatively with a return to the pre-operative state at 12-months. Additionally, anterior stromal oedema with hyper-reflective cytoplasm and extracellular lacunae in a honeycomb-like appearance may be observed for up to 3 months postoperatively. Obviously, potentially cytotoxic effects of CXL on the corneal endothelium is a major concern, however, it appears that this does not occur as long as a minimum corneal thickness of >400µm is adhered to.

A-CXL with intensities of 18 mW/cm² for 5 minutes and 30 mW/cm² for 3 minutes are reported to have similar microstructural effects as the Dresden protocol, though there is some indication that the anterior microstructural changes are more pronounced, however, a return to baseline cell densities is reported at 12-months.

In this study, the corneal basal epithelial, posterior keratocyte and corneal endothelial cell densities were unaffected by the CXL procedures performed, while the corneal sub-basal nerve plexus density was reduced at 1, 3 and 6 months, returning to baseline levels at 12-months. All these observations are consistent with previous investigations that utilised an intensity of 30 mW/cm². However, the anterior keratocyte density remained significantly reduced at 12-months in this study, though there was a trend toward returning to baseline levels. Therefore, it is possible that the higher irradiance (7.2 J/cm²) has a greater effect on the anterior keratocytes than the traditional 5.4 J/cm², resulting in a longer recovery period for anterior stromal keratocytes. However, longer follow-up beyond 12 months would be required to determine if, and when, anterior keratocyte density returns to baseline levels.

Interestingly, the only other published report utilising the same A-CXL protocols as the current study suggested that (normal) keratocyte repopulation occurred 6 months post-operatively. However, the study was relatively small, with only 10 eyes in each group and the authors did not disclose the method by which anterior keratocyte density was determined.

Treatment with CXL often results in a modest improvement in UCVA and BSCVA as well as SE. For the Dresden protocol, at 12-months an improvement of approximately 2 lines in UCVA and BSCVA and an improvement of 0.65D in SE has been reported. The Dresden, 9 mW/cm², 18 mW/cm² and 30 mW/cm² intensities, yielded similar differences in BCVA and SE when post-operative improvements in both parameters were compared between intensities. Interestingly, the CA-CXL and PA-CXL accelerated high intensity UV-A protocols used in this study produced improvement
of approximately 2 lines in UCVA and 1 line in BCVA in the CA-CXL group and 1 line in UCVA and BCVA in the PA-CXL group at 12-months. Additionally, there was an improvement of approximately 1.5D in SE at 12-months in both groups. It is important to note that the changes in UCVA, BCVA and SE were only significantly different within the groups, there was no statistical difference in these changes between the groups.

Much like measuring progression in keratoconus, measuring the efficacy of CXL is challenging. The measures of a successful outcome are generally based on whether there is progression in corneal shape, refraction and visual acuity. The greatest emphasis is placed on changes in anterior corneal power/shape. A recent Cochrane review revealed that at 12-months, the mean maximal keratometry was 1.92D less than the preoperative state in the studies included in the meta-analysis. There was a significant reduction in the risk of progression (defined as an increase in maximal keratometry of 1.5D or more at 12-months following treatment), with a relative risk of 0.12. However, at 36 months post-operatively, with progression defined as an increase in K_max of 2.0D or more in this period, the relative risk is even lower at 0.03.

Progression despite treatment with CXL (termed treatment failure) has been reported to occur in 4.8% and 7.6% of cases. Variations in the rates of treatment failure between studies are likely the result of different definitions of treatment failure. Investigations with UV-A intensities of 6 mW/cm², 9 mW/cm², 18 mW/cm² and 30 mW/cm² compared to the Dresden protocol reveal flattening of the cornea at 12-months in all cases. However, it appears that the flattening effect may reduce as the intensity of UV-A increases. Mean K_max at 12-months was reduced by 1.32D (Dresden protocol), 1.23 (6 mW/cm²), 0.67D (9mW/cm²), 0.52D (18 mW/cm²) and 0.18D (30 mW/cm²). Treatment failure rates of 3% were noted for the 18 mW/cm² and 30 mW/cm² groups.

The results of this study suggest that there is no mean change in K_max, K_steep, K_flatt, CCT or TCT at 12-months post-operatively. In contrast, a recent investigation utilising the same treatment protocol as the current study reported a significant mean reduction in maximum keratometry of 1.4D in the PA-CXL group but no change in the CA-CXL group, claiming that the PA-CXL group had greater flattening. However, the authors compared absolute values not differences which is an important distinction as the CA-CXL group appears to have greater disease severity at baseline, and the study is likely under-powered with only 10 eyes in each treatment group. Of note, 26 (66%) eyes in the current study that had 12-months follow-up (65% CA-CXL, 63% PA-CXL) showed ≥0.2D of flattening at 12-months, similar to other investigations utilising UV-A of 30 mW/cm². It is noteworthy that the current study did not exclude participants based on maximum keratometry, which is generally an
exclusion criterion due to CXL being less effective in cases of more severe keratoconus. This study aimed to treat all participants who met the safety limitations of corneal thickness as ultimately CXL is aimed at preventing disease progression and corneal transplantation, thus should be implemented in all cases of progressive disease where possible. Indeed, 36% of all treated eyes had a maximum keratometry ≥60D, which may account for the lack of a reduction in the mean K max. Additionally, only 2/3 of treated eyes had reached the 12-month follow-up which may have skewed the results.

Anterior segment OCT enables imaging of the apparent demarcation between treated and untreated corneal stroma. Though not definitively established, it is believed the demarcation line indicates how deep the cross-linking effect has occurred. This demarcation line is generally quantified as an absolute value of the depth at which it occurs in the cornea. When the Dresden protocol is utilised, the depth of the demarcation is usually around 300µm. A-CXL treated corneas exhibit a shallower demarcation. It appears that with increasing intensity, the demarcation line is shallower and more variable. With the 18 mW/cm² and 30 mW/cm² intensities the average depth of demarcation has been reported at 203µm and 184-201µm, respectively.

Absolute measures may not necessarily be the optimal method of assessing the depth of CXL demarcation and the percentage of treated cornea may prove to be a better indicator of treatment success. In this respect, the demarcation line results of this study are not directly comparable to those of other investigations of CXL. However, average percentage depth of demarcation can be produced utilising the CCT and depth of demarcation from other investigations. The Dresden protocol had a greater percentage depth of demarcation (73.0%) than the 9mW/cm² (40.8 %) and 30 mW/cm² (57.2%) treatment groups, as expected. Though these calculations utilised the baseline mean CCT and demarcation at 1 month and has inherent limitations in that CCT was obtained from tomography and demarcation from OCT. Nonetheless, the percentage depth of demarcation in our study is similar to the 30mW/cm² study at 1 month (57.2%) in both the CA-CXL and PA-CXL groups (53.0% and 52.6%, respectively). Interestingly, a recent study of the demarcation line produced using the same protocol as the current study suggested that the demarcation line is significantly deeper in the PA-CXL treatment group (201.1 vs. 159.9 µm, respectively).

In vivo assessment of the biomechanical properties of the cornea is a relatively recent development, which has the potential to quantify the stiffening effect of CXL on the cornea which has been demonstrated ex vivo. However, recent attempts to corroborate the ex vivo results using devices such as the CorVis ST and Ocular Response Analyser (ORA, Reichert Inc., Depew, NY, USA) in vivo, have not demonstrated a significant increase in corneal stiffness post CXL. The results of the current investigation which utilised the deformation amplitude provided by the CST, showed no
change in the DA at any time point post-operatively, similar to a previous investigation utilising 30 mW/cm². However, it has been noted that the DA output provided by the CST is actually a combination of corneal deformation and inward movement of the entire globe under the influence of the air-pulse. The corneal deformation component as well as other parameters, such as dynamic curvature, can be obtained through external analysis of the video sequence captured by the CST and ultimately analyses of these parameters may reveal an improvement in biomechanical integrity post CXL but are beyond the scope of this investigation.

Considering the often slow progressive nature of the keratoconus disease process, the major limitation of most investigations of A-CXL is the relatively short term follow-up period. Indeed, this is mainly due to A-CXL having only come into use recently, while investigations utilising the Dresden protocol have been carried out for over a decade. Thus investigations of the Dresden protocol with 7 and 10 years of follow-up have emerged recently; the results of which suggest that the improvement in KMax and vision are maintained over the entirety of the studies follow-up. In stark contrast, the majority of investigations of A-CXL have follow-up periods of 12, 15, 18 and 24 months. Therefore, currently judging the efficacy of these accelerated protocols is difficult as their very long term results are not yet available.

The shallower demarcation line, lower amount of corneal flattening and improvements in UCVA, BSCVA and SE in accelerated cross-linking, compared to the Dresden protocol, may be indicative of fewer cross-links being formed, the amount of which are apparently inversely proportionate to the intensity of UV-A used. As previously noted, oxygen is essential to the cross-linking process in order for the type II photodynamic reaction to occur. In the case of A-CXL, the irradiation time is reduced by increasing the intensity of the UV-A to maintain the same total amount of energy.

As previously discussed, higher intensity UV-A depletes oxygen at a higher rate in A-CXL, which in combination with reduced irradiation time, results in less oxygen availability and less oxygen diffusion into the cornea over the treatment period, resulting in a predominantly type I reaction. In contrast the longer irradiation time of the Dresden protocol may allow for greater oxygen diffusion and a greater proportion of type II reaction. To over-come these limitation it was proposed that the UV-A be pulsed at a rate of 0.5Hz with higher intensity (30 mW/cm²) and higher irradiance (7.2 J/cm²) to improve the efficacy of A-CXL by allowing a greater amount of oxygen to diffuse into the cornea in the interim second between “pulses”.

Interestingly, the results of the current studies suggest that the theoretical benefit of pulsed CXL may not be reflected in measurable clinical outcomes. Indeed, the CA-CXL group, effectively a control
group to determine if pulsing UV-A had a significant effect, demonstrated the same clinical efficacy as the PA-CXL group. None-the-less this may reflect the reduced total duration over which the UV-A irradiation occurs and the length of the pulse intervals where there is no UV-A exposure (1s) may be insufficient for significant oxygen diffusion. Additionally, the CA-CXL and PA-CXL protocols utilised riboflavin soak time was 1/3 that of other studies (10 minutes vs. 30 minutes). The riboflavin soak was reduced to 10 minutes in this study as per the guidelines of the manufacturer (Avedro), who report that this is sufficient for their proprietary VibeX Rapid riboflavin formulation to achieve adequate saturation and penetration.

9.5 Conclusions

The results of this study suggest that both the continuous and pulsed UV-A forms of the protocol are safe and effective treatments for keratoconus. These treatment protocols have similar complication and failure rates to previously established A-CXL protocols and to the original Dresden protocol. Furthermore, the microstructural changes are similar to the aforementioned studies, with the exception of the anterior keratocytes which may take longer to recover than observed in other protocols.

In terms of efficacy, the results suggest that both the continuous and pulsed methods are effective at halting disease progression at 12-months post-operatively with no difference between the two groups. However, both the continuous and pulsed treatment groups did not produce significant flattening of the cornea. However, longer term follow-up will ultimately reveal the efficacy of these treatment protocols in this and other studies.

The results suggest that the continuous treatment protocol may be preferable as the UV-A irradiation time is half that of the pulsed protocol and that there was no significant difference between the continuous and pulsed treatment protocols in terms of complication and failure rates and changes in measures of efficacy, though the continuous treatment group had slightly better improvement in UCVA and BCVA at 12 months.
Section 5:

Summary of Aims, Methodology, Results, Discussion and Conclusions

Chapters 10-11
Chapter 10:

Summary of Aims, Methodologies and Key Results
**10.1 Introduction:**

As highlighted throughout this thesis, there is a distinct lack of investigations of the epidemiology, clinical characteristics and natural history of keratoconus in NZ considering how common the condition appears to be. Furthermore, NZ has amongst the highest proportions of corneal transplantations performed for keratoconus worldwide, hence subjects with keratoconus in NZ may benefit immensely from CXL. The apparently high prevalence of keratoconus in NZ also makes it an ideal location to investigate methods of improving the efficiency of CXL as a great number of individuals may require the procedure in the coming years.

The ARK project that forms the basis of this thesis consisted of six inter-related studies that were focused on the clinical aspects of keratoconus that have not necessarily received enough attention in NZ. The studies detailed in chapters 4-7 focused on the epidemiology, clinical characteristics and natural history of keratoconus, while those detailed in chapters 8-9 focused on the repeatability of computerised tomography devices in keratoconic eyes and the safety and efficacy of two accelerated CXL (A-CXL) protocols. Each chapter contains detailed accounts of each study, thus this penultimate chapter provides a summary of the aims, methodology and key results of each of these studies.
10.2 Aims, Methodology and Key findings of Chapters 4-9

10.2.1 Chapter 4 – The Epidemiology, Demographics, Distribution and Basic Clinical Characteristics of Subjects with Keratoconus in New Zealand – The Aotearoa Research into Keratoconus (ARK) Study: Part I

The purpose of the ARK Study: Part I was to investigate the epidemiologic, demographic and basic clinical characteristics of individuals with keratoconus managed by optometrists in NZ/Aotearoa. A prospective, longitudinal, nationwide, survey protocol was completed for every patient with keratoconus that underwent a consultation with participating optometrists in a 2-year period. Data for each patient included: date of birth, gender, self-reported ethnicity, new or prior diagnosis, UCVA, BCVA, type of refractive correction required to obtain BCVA and keratometry.

A total of 1869 cases were identified; mean age 41.0 ± 15.7 years, 56.4% male, 87.3% with a prior diagnosis. The distribution of cases was skewed toward Auckland (41.6%), Waikato (21.3%), Wellington (16.8%) and Bay of Plenty (13.3%). Self-reported ethnicities were predominantly NZ European (54.4%), Māori (24.7%), and Pacific Peoples (15.5%), disproportionate to the general population profile (66.7%, 13.5%, and 6.6% respectively). Most eyes (64.3%) were managed with rigid contact lenses (corneal lens in 34.2%). The mean K\textsubscript{mean} was 49.0 ± 5.7D. The mean UCVA was 6/42 and BCVA was 6/9. Māori and Pacific Peoples had both the highest K\textsubscript{mean} and proportions of eyes graded stage IV and lowest proportions graded stage I-II on the Amsler-Krumeich scale.

The results of this study indicate that keratoconus is relatively common in NZ with at least 1869 patients managed by optometrists in 2 years. The majority of eyes had mild-moderate disease; however, Māori and Pacific Peoples appeared to have greater disease severity. An ethnic predilection was apparent, with Māori and Pacific Peoples over-represented relative to their population proportions, reinforcing a long-held clinical suspicion and possibly indicating a need for targeted screening of these populations in order to identify keratoconus earlier and offer appropriate interventions such as CXL.
10.2.2 Chapter 5 – The Natural History and Severity of Keratoconus in Participants with a Neophyte Diagnosis in the Auckland Region – The Aotearoa Research into Keratoconus (ARK) Study: Part IIA

The ARK Study: Part IIA was a prospective longitudinal investigation intended to characterise the corneal phenotypic features of keratoconus at initial diagnosis in NZ and track changes in these features as the disease progressed using advanced anterior segment imaging including corneal tomography, anterior segment OCT, in vivo confocal microscopy and corneal biomechanical analysis. Participants with a new diagnosis of keratoconus were to undergo a baseline visit and two follow-up visits 8 months apart. These results would in turn also allow for the comparison of disease characteristics/severity and rates of progression between ethnicities in NZ and determine the order in which changes in corneal tomography occur relative to changes in refractive error, vision, corneal microstructure and biomechanics.

Unfortunately, this study had to be halted, with all follow-up discontinued after the baseline visit of 30 participants because 67% of participants had received CXL in at least one eye following their baseline visit and most subjects being referred to the study requiring or being eligible for CXL which could not ethically be withheld. Nonetheless, analyses of the 30 participants suggested that keratoconus is typically asymmetric in terms of $K_{\text{flat}}$, $K_{\text{steep}}$, $K_{\text{mean}}$, $K_{\text{max}}$ and DA being higher and CCT, TCT, sub-basal nerve density, and anterior and posterior keratocyte density being lower in the worse eye. As expected, the worse eye also had poorer UCVA and BSCVA. Correlation between corneal power/shape and thickness and refractive error, vision, microstructure and biomechanics suggest that several parameters utilised in combination may provide a more comprehensive means of assessing progression. However, the inability to obtain longitudinal follow-up data limits the applicability of the results.

This study concluded that keratoconus may be more asymmetric in NZ than other parts of the world and that investigations of the natural history of keratoconus utilising up to date technological advances are still necessary and valuable in further developing the risk profile of progressive keratoconus. However, with CXL becoming standard of care in patients with progressive disease, prospective studies of the natural history of keratoconus will likely become increasingly difficult to conduct and may limit future investigations to retrospective analyses.
10.2.3 Chapter 6 – Assessment of the Natural History of Keratoconus through Retrospective, Longitudinal Analysis of Contact Lens Clinical Records – The Aotearoa Research into Keratoconus (ARK) Study: Part IIB

As a response to the inability to complete ARK Study Part IIA, as noted in the preceding section, the ARK Study Part IIB (detailed in chapter 6) was designed to assess the natural history of corneal curvature/power and CLVA of subjects with keratoconus via retrospective, longitudinal analysis. The contact lens clinical records of two specialist optometry practices were analysed to extract data at yearly intervals concerning CLBC, CLVA, K_{steep}, K_{flat} and K_{mean}.

In subjects with keratoconus the CLBC continued to increase significantly up until the age of 40 – 50 years, suggesting that corneal curvature/power continues to increase up until at least this age. However, a significant reduction in the mean CLVA was only observed once the CLBC being utilised increased to >48.0D, and the mean CLVA continued to reduce significantly as CLBC increased further above 48.0D. An increase in K_{steep}, K_{flat} and K_{mean} above 48D was also suggestive of a similar reduction in CLVA. Higher corneal curvature/power as indicated by CLBC, K_{steep}, K_{flat} and K_{mean} increased the odds of a CLVA less than 6/12, with a CLBC >48.0D (<7.0mm) carrying the greatest increased odds (OR 3.66).

Despite limitations due to the retrospective nature of this study, it provided considerable insight into the natural history of keratoconus, particularly the longitudinal changes that occur in corneal curvature/power and how disease severity in terms of corneal and curvature/power relates to CLVA.
10.2.4 Chapter 7 – The Natural History of Corneal Topographic Progression of Keratoconus after Age 30 years in Non-contact Lens Wearers – The Aotearoa Research into Keratoconus (ARK) Study: Part IIC

The ARK Study Part IIC was developed in light of the observation in the ARK Study Part IIB (chapter 6) that keratoconus appeared to continue to progress beyond age 30 years. The investigations detailed in chapter 7 therefore aimed to determine if significant progression of the disease occurs in older, non-contact lens wearing, subjects with keratoconus and to identify potential predictive factors. Clinical and computerised corneal topography records of subjects with keratoconus attending a specialist optometry practice were retrospectively analysed to identify suitable participants. The following topographic parameters were assessed at the first (baseline) consultation and most recent (final) examination: K_max, K_steep, K_flat, I-S ratio, and the surface asymmetry and regularity (SAI, SRI) indices.

Forty-three eyes of 27 participants met the inclusion criteria, with a median age of 38.45 (12.86) years at baseline and median follow-up of 4.36 (8.68) years. There was a significant increase in median K_max (0.30 (1.21) D), K_steep (0.27 (0.90) D), K_flat (0.34 (1.12) D) and I-S (0.26 (0.82) D) between baseline and final review, p<0.05. Notably, 18.6%-25.6% of eyes demonstrated ≥1.00D increase in one or more of four principle topographic parameters (K_max, K_steep, K_flat, I-S ratio) while 18.5%-37.0% of subjects had ≥1.00D increase in the aforementioned parameters in at least one eye over the study period. However, <10% of eyes exhibited ≥1.00D increase/year in all topographic parameters. Ultimately, the only significant predictor of progression was length of follow-up.

This study confirmed that keratoconus may continue to progress beyond the fourth decade and older subjects with keratoconus should be monitored for progression, particularly with respect to possible CXL and astigmatic correction in cataract surgery.
10.2.5 Chapter 8 – Repeatability and Agreement of Orbscan II, Pentacam HR and Galilei Tomography Systems in Corneas with Keratoconus – The Aotearoa Research into Keratoconus (ARK) Study: Part IIIA

The aims of the study detailed in chapter 8 were to assess the repeatability and agreement of keratometry and pachymetry measurements obtained using the Orbscan II, Pentacam HR and Galilei G2 Tomography Systems in corneas with Keratoconus. Fifty eyes of 50 participants with keratoconus were scanned with all three devices to obtain measurements of $K_{\text{steep}}$, $K_{\text{flat}}$, CCT and TCT. Repeatability of these measurements were assessed using the within-subject standard deviation ($S_w$), coefficient of variation (CV) and intraclass correlation coefficient (ICC), and comparability between device pairs was assessed using Bland-Altman plots.

For all studied parameters, ICC was $>0.98$ with the least repeatable measurements obtained using Orbscan II. Mean $K_{\text{steep}}$ values were similar while mean $K_{\text{flat}}$ values were significantly different between all devices. The Galilei G2 and Pentacam HR had the lowest 95% limits of agreement for both CCT and TCT. There were no significant differences in mean CCT between Galilei G2 and Pentacam HR ($p=0.10$) although Galilei G2 measured higher TCT (7.4µm, $p<0.001$). Mean Orbscan II CCT measurements were higher compared to Pentacam HR (10.6µm, $p=0.027$) and Galilei G2 (8.3µm, $p=0.071$), but significantly lower when an acoustic factor of 0.92 was applied (-28.9µm vs. Pentacam HR, $p<0.001$) and (-31.2µm vs. Galilei G2; $p<0.001$).

This study suggests that keratometric and pachymetric measurements of keratoconic eyes obtained by Galilei G2, Orbscan II, and Pentacam were disparate. Measurements were less repeatable with Orbscan II compared to Pentacam HR and Galilei G2 although overall repeatability was high for all instruments.
10.2.6 Chapter 9 – Safety and Efficacy of High Intensity, High Irradiance Accelerated Corneal Collagen Cross-linking with Continuous and Pulsed Ultraviolet Exposure – The Aotearoa Research into Keratoconus (ARK) Study: Part IIIB

The study outlined in chapter 9 was purposed to investigate the safety and efficacy of two novel protocols for accelerated CXL, which entailed using high intensity, high irradiance UV-A. In one protocol the UV-A was continuous (CA-CXL) and in the other it was pulsed (PA-CXL). The two protocols utilised UV-A with a total irradiance of 7.2 J/cm² and intensity of 30 mW/cm². Participants were randomized to receive one of the two different UV-A protocols and examinations were performed pre-operatively and 1, 3, 6 and 12-months post-operatively. Data regarding vision and refractive error, corneal tomography, anterior segment OCT, in vivo confocal microscopy and corneal biomechanics were collected at each examination.

CA-CXL was performed on 29 eyes and PA-CXL on 28 eyes. The results of this study suggested that both CA-CXL and PA-CXL were safe as there were no post-operative infections or infiltrates, similar rates of corneal haze/scarring and treatment failure compared to previous investigations, no change in posterior keratocyte, corneal endothelial cell and epithelial cell density post-operatively, and similar recovery of corneal sub-basal nerve density at 12-months as described in previous investigations. However, the anterior keratocyte density may take longer to recover than observed in other protocols, remaining reduced 12-months post-operatively.

In terms of efficacy, the results suggest that both the continuous and pulsed methods are effective at halting disease progression at 12-months post-operatively with no significant change in $K_{\text{steep}}$, $K_{\text{flat}}$, $K_{\text{max}}$, CCT, TCT and DA. Furthermore, there was no difference between the change in these parameters between the CA-CXL and PA-CXL groups. UCVA and BCVA showed no significant change but trended toward improvement, while SE improved significantly 12-months post-operatively with no difference between the CA-CXL and PA-CXL groups. The results of this study revealed that the measured parameters of keratoconus stabilized in both the CA-CXL and PA-CXL groups, although longer term follow-up will ultimately reveal the efficacy of these protocols over an extended period.

This study concluded that the CA-CXL treatment protocol may be preferable given that the UV-A irradiation time is half that of the PA-CXL protocol and that there was no significant difference between the continuous and pulsed treatment protocols in terms of complications, failure rates or changes in measures of efficacy.
Chapter 11:

Discussion and Conclusions
11.1 Introduction
The six inter-related studies that make up the ARK project and are the basis of this thesis focused on the epidemiological and clinical phenotypic characteristics of keratoconus in NZ (chapters 4-8) and the safety and efficacy of two novel accelerated corneal collagen cross-linking protocols for the treatment of progressive keratoconus (chapter 9). The results of the study detailed within each chapter were compared to those of similar investigations and their over-arching implications for their specific areas of the literature were explored within the discussion section of each chapter. This final chapter discusses the results of the studies detailed in chapters 4-9 in relation to each other and their wider implications for the current state of our knowledge of keratoconus in NZ, the literature, clinical practice and future investigations.

11.2 Discussion
The ARK Study: Part I (ARK Part I) is the largest epidemiological study of keratoconus that has been conducted in NZ to date. Furthermore it was carried out at a primary care level, aggregating data from optometrists. This methodology resulted in a sample size of 1869 cases, collected from around the country. Hence the results of the investigation have potential implications for clinical practice across NZ as a whole. Indeed, ARK Part I has confirmed the long standing clinical impression that keratoconus may have an ethnic predilection for the Māori and Pacific Peoples. Additionally, ARK Part I suggested that keratoconus is in fact not only more common among the Māori and Pacific Peoples ethnicities but potentially also more severe. Hence the suspicion of keratoconus in individuals of Māori and Pacific Peoples ethnicities should be raised, particularly in teenage individuals, in order to increase the likelihood of detecting the condition at an early stage. Modifying clinical practice in this way may be particularly important for primary health care providers such as optometrists who are most likely to encounter individuals experiencing visual impairment without other symptoms and thus may have early keratoconus.

Ethnic disparity in health outcomes is a global issue and NZ is no exception. It is well documented that individuals of Māori descent have worse health outcomes, compared to other ethnicities, in a multitude of conditions including; breast cancer, prostate cancer, cardiovascular disease and stroke. Ethnicity based inequality in health outcomes in NZ is a complex issue with myriad underlying influences including: disparity in access to healthcare, cultural perceptions about health care, socioeconomic status and ethnic bias in clinical decision making.

It is unclear if there is an inequality in the health outcomes of individuals with keratoconus who are of Māori and Pacific Peoples descent. Indeed, as discussed in chapter 4, NZ has amongst the highest
proportions of corneal transplantations performed where the indication is keratoconus, but the ethnic make-up of the individuals undergoing these transplantations is unclear.\textsuperscript{40, 41} Ethnic variation in receiving a corneal transplantation for keratoconus in itself may not be a worse health outcome, it may instead be an indicator of ethnic variation in disease severity and progression (discussed later). However, impaired vision as the result of a failed/rejected graft can be considered a worse health outcome. At present, there have been no investigations of the ethnic variations in corneal transplantation survival in NZ, however, a recent study of appointment attendance as a surrogate measure of post-operative treatment adherence in corneal transplantation recipients in Auckland, NZ, revealed that Māori and Pacific Peoples ethnicities were significantly associated with a lower rate of appointment attendance,\textsuperscript{329} which may be indicative of a potential ethnic disparity in corneal graft survival.

A recent investigation on the effect of corneal collagen-crosslinking (CXL) on keratoplasty rate has demonstrated that six years following the implementation and establishment of CXL, the proportion of keratoplasties performed for keratoconus was reduced to <50% of that of the year preceding the establishment of CXL.\textsuperscript{185} Thus, in the near future, ethnic disparity in keratoplasty rates for keratoconus may be considered a disparity in health outcome and emphasises the need for early disease detection in order for the implementation of CXL to have the greatest success.

As suggested in chapter 4, the results of ARK Part I suggest that a targeted screening program of Māori and Pacific Peoples ethnicities for keratoconus, particularly in the teenage years, is a possible avenue to explore in order to detect the condition earlier and offer appropriate interventions such as CXL. A possible route through which this could be advanced is high school screening. Indeed, in NZ it is a well-documented, unfortunate, issue that individuals of Māori and Pacific Peoples ethnicities tend to suffer lower socioeconomic status, having the lowest proportions of adults earning <$10,000 a year and highest rates of unemployment,\textsuperscript{A} thus tending to reside within the similar (socio-economic) areas. As a result their children attend the same schools, an observation previously used to effect in the investigation of the ethnic variation in keratoconus in teenagers in NZ by Owens et al.\textsuperscript{188} Screening for keratoconus in this way may have the added benefit of screening for general visual impairment in ethnic groups that may not otherwise receive routine eye-care, particularly since optometry is currently a relatively costly, private-only, healthcare service in NZ and children only receive a general visual screening prior to starting primary school,\textsuperscript{330} typically prior to the usual onset of keratoconus.

Although ARK Part I is the largest epidemiological study of keratoconus in NZ to date, only 10.5% of optometrists were surveyed. Thus as mentioned in chapter 4, the number of individuals with
keratoconus captured by the survey is likely a significant underestimate of the total population of individuals with keratoconus in NZ. Hence a meaningful prevalence estimate of keratoconus in NZ could not be made; however, the fact that approximately 10% of participants had a new diagnosis of keratoconus provides an indication of relative incidence which can be utilised in planning current and future resource allocation to treat keratoconus.

The author believes that ARK Part I represents another step in the journey to determining the prevalence of keratoconus in NZ and future investigations will certainly be a challenge as they will need to encompass practically all individuals with keratoconus, thus will need to include both ophthalmologists and optometrists, as well as account for the overlap of subjects managed by both. A possible means to accomplish this would be a universal keratoconus registry, utilised by both ophthalmologists and optometrists. However, in addition to potential ethical issues in accumulating such data on a national scale, this would also be a considerable administrative challenge in terms of cost to set up and maintain, not to mention ensuring that ophthalmologists and optometrists enter the pertinent data for all/most subjects.

As previously mentioned, it is not entirely clear why the proportions of keratoplasties performed where the indication is keratoconus is higher in NZ compared to other parts of the world. It is possible that this increased transplantation rate for keratoconus is an indicator of ethnic variation in disease severity and/or progression. ARK Part I did show that keratoconus is possibly more severe in individuals of Māori and Pacific Peoples ethnicities; however, there were some limitations in the way disease severity was measured, primarily that K readings were obtained from different keratometry and computerised topography devices.

The ARK Study Part IIA (Chapter 5) was intended to prospectively, longitudinally track changes in the phenotypic characteristics of the cornea as keratoconus progresses in a large cohort, but was unexpectedly cut short due to a large proportion of participants undergoing or requiring CXL. Indeed, ARK Part IIA had the potential to characterise ethnic variations in the severity and rates of progression of keratoconus, especially considering that the ethnic make-up of the cohort was already shaping up to reflect the ethnic bias of the disease overall with the Māori and Pacific Peoples ethnicities being over-represented at the time the study ceased (approximately 15% and 50% of the cohort respectively). While this study could not be completed, analysis of the available baseline data did reveal that the inter-eye tomographic disease severity may be greater in NZ than in other parts of the world. It is possible that the greater inter-eye asymmetry in disease severity may lead to later presentation by subjects, with greater disease severity in the more affected eye being tolerated by
the subject for longer as the less severe eye may provide adequate overall vision. This highlights the need for early disease detection.

The other aim of ARK Part IIA was to provide an update on the natural history of keratoconus utilising modern anterior segment imaging techniques, characterising the longitudinal changes that occur in computerised corneal tomography and how these relate to changes in vision, refractive error, microstructure and biomechanics. Thus ARK Part IIA was intended to be a modern update on the CLEK\textsuperscript{2} and DUSKS\textsuperscript{3} studies. Additionally, ARK Part IIA had the potential to elaborate on the disease risk profile of keratoconus in terms of how disease progression is related between eyes at an individual level. Indeed, all eyes of the participants were considered separately in the analyses carried out in chapters 4, 6, 7 and 9, as keratoconus is generally asymmetric in its inter-eye presentation. Specifically, averaging of the eyes or utilising just one eye in keratoconus subjects has the potential to create a bias in disease severity. However, assessment of each eye separately also has limitations since one eye from any individual is highly unlikely to behave entirely independently of the contralateral eye.

Notably, the CLEK study specifically characterised the likelihood of disease progression in each eye and in at least one eye of an individual, based on individual and eye specific characteristics such as: age, gender, disease severity in terms of corneal curvature and scarring and contact lens use.\textsuperscript{251, 253, 257, 264} Other studies have attempted to characterise individuals who will progress from normal or keratoconus suspect to manifest keratoconus,\textsuperscript{68, 224, 239} however, the progression of keratoconus, in terms of how it is related between the eyes of the same individual, is still poorly characterised. ARK Part IIA was designed with the potential to shed some light on the relationship of disease progression of each eye within the same individual, had the longitudinal assessment not been curtailed. Therefore, this remains an area of keratoconus study that requires further enquiry, particularly to inform the ocular risk profile of individuals who require CXL – specifically in terms of whether the procedure should be performed unilaterally or bilaterally.

The fact that ARK Part IIA could not be completed, as most participants received CXL following their baseline visit or were eligible for CXL, highlights that prospective longitudinal investigations of keratoconus may no longer be possible in NZ or elsewhere. Thus the natural history of keratoconus may need to be ascertained through retrospective analyses. This led to the development of the ARK Study: Parts IIB and C reported in chapters 6 and 7 respectively.

Both ARK Part IIB and C were retrospective, longitudinal investigations of the natural history of keratoconus, and both studies revealed that keratoconus may continue to progress beyond age 30
years in some cases, suggesting that individuals with keratoconus may need to be monitored for progression beyond the fourth decade. This has implications for potential use of CXL at an older age and consideration of astigmatic correction in cataract surgery. Interestingly, both ARK Part IIB and C consolidate the observations of the CLEK study, which reported that while the rate of progression reduces with age, keratoconus continues to progress beyond age 30.  

ARK Part IIB also characterised the relationship between corneal curvature/power and contact lens corrected visual acuity (CLVA) through the analysis of contact lens base curve (CLBC) and keratometry. As previously noted this approach has the potential to inform both practitioner and patient of the potential CLVA that may be attained with a given CLBC, keratometric values, or change in these parameters. As highlighted in their respective chapters both ARK Part IIB and C were limited to assessment of anterior corneal parameters and thus did not assess changes in corneal thickness and posterior elevation, which highlights the need for computerised corneal tomography (3-dimensional) based investigations. However, older subjects with keratoconus are generally not followed-up in a tertiary care in NZ, where the majority of computerised corneal tomographers are located. This poses limitations on retrospective investigations concerning these older subjects.

The current definitions for progression in keratoconus are generally based on changes in vision, refraction, corneal curvature/power and corneal thickness, as was the case in the investigation of CXL in chapter 9 of this thesis. However, the most common definitions of progression are based on changes in computerised corneal tomographic parameters, such as simulated keratometry and corneal thickness. These criteria for progression may be the most accurate as they are generally based on the repeatability of the computerised corneal tomography device.

As detailed in the studies in chapter 8 (ARK Part IIIA), the repeatability of computerised corneal tomographic parameters in eyes with keratoconus, pertaining to simulated keratometry and pachymetry, varies considerably between devices e.g. the “repeatability” of $K_{\text{steep}}$ was poorer with the Orbscan II, 2.25D, compared to the Pentacam HR, 1.00D. Furthermore, ARK Part IIIA revealed that $K_{\text{steep}}$, $K_{\text{flat}}$, CCT and TCT cannot be used interchangeably between the Orbscan II, Pentacam HR and Galilei G2 devices in eyes with a keratoconus. This remains a common conclusion drawn from investigations of the repeatability and comparability of different tomography devices on normal and keratoconic eyes.

The variation in repeatability and lack of comparability between different tomography devices suggests that the criteria for progression in keratoconus should be based upon the repeatability of the particular device being used. Specifically, these criteria cannot be applied when scans from
different devices on the same individual are compared. An advantage of using the repeatability of a
device to define progression in keratoconus is that it has the potential to create uniform definitions
of progression from a secure evidence base. This makes studies of the progression of keratoconus or
the efficacy of CXL more comparable, as opposed to the multiple variations in criteria for progression
or treatment failure, currently used. Therefore, it is important that the repeatability and
comparability of the many tomography devices available be determined on eyes with keratoconus in
order to produce appropriate definitions of progression and to ascertain how readily scans from
different devices can be reliably compared.

As stated in chapter 8 (ARK Part IIIA) the investigation of the repeatability and comparability of the
Orbscan II, Pentacam HR and Galilei G2 was initiated following the recruitment of participants for the
CXL investigation detailed in chapter 9 (ARK Part IIIB). All participants had initially been examined
using the Orbscan II (with acoustic factor of 0.92 for corneal thickness measurements) prior to being
referred to the specialty CXL clinic. It was immediately noted that these pachymetry measurements
were significantly lower than those obtained with the Pentacam HR. The criteria for progression
based on tomographic parameters utilised in ARK Part IIIB were in place prior to carrying out these
repeatability analysis; however, it appears that these criteria are corroborated by some of the
repeatability results. Indeed, the repeatability for TCT for the Pentacam HR was 28.1µm, while the
criterion for progression was a reduction in corneal thickness of ≥30µm.

Corneal collagen cross-linking was relatively recently developed to slow down or halt the progression
of keratoconus, with the first clinical account being published just over a decade ago. Thus, there
have been numerous studies into improving the safety, efficacy and efficiency of the procedure. To
improve the efficiency of CXL, UV-A of higher intensity has been utilised to reduce the amount of
time required to carry out the procedure. Therefore, ARK Part IIIB was designed to study the safety
and efficacy of two novel protocols of accelerated CXL (A-CXL), utilising a shorter riboflavin soak time
with a proprietary riboflavin solution produced by Avedro (Avedro Inc., Waltham, MS, USA), higher
total energy (7.2 J/cm² vs. 5.4 J/cm²) and intensity (30 mW/cm²) of UV-A irradiation and either pulsed
(PA-CXL) or continuous (CA-CXL) UV-A. The results of ARK Part IIIB suggest that both the CA-CXL and
PA-CXL protocols are effective at halting the progression of keratoconus and there appears to be no
significant difference in this effect between the two protocols. However, as noted in chapter 9, both
these treatment protocols and other protocols of A-CXL produced less reduction in corneal
curvature/power, shallower demarcation lines, less improvement in UCVA, BSCVA and SE than the
original Dresden protocol in an apparently inverse relationship to the intensity of UV-A.
Thus is it possible that improving the efficiency of CXL by utilising accelerated protocols may result in a less obvious change in measured clinical parameters. However, as previously noted, a lack of improvement in corneal curvature/power, UCVA, BSCVA and shallower demarcation, are not necessarily a failure of the treatment as CXL is aimed at preventing progression, which several different A-CXL protocols appears to achieve and sustain over 1-2 years. Importantly, the Dresden protocol has now been shown to be effective over 7-10 years and can reduce the transplantation rate for keratoconus. Ultimately, time will tell if the various modified A-CXL protocols, including those outlined in ARK Part IIIB, are equally effective over a longer period and are capable of being more efficiently utilised for treatment in place of the Dresden protocol.

As noted in ARK Part IIIB, a possible contributor to the lack of a reduction in corneal curvature/power following the A-CXL protocols is that this study aimed to treat all participants who met the safety limitations of corneal thickness, thus a significant proportion of eyes had more severe disease. This has been shown to reduce the flattening effect of CXL (36% of all treated eyes had a maximum keratometry ≥60D). Higher $K_{\text{max}}$ was not utilised as an exclusion criteria in ARK Part IIIB as CXL is aimed at preventing disease progression, thus maintaining subjects in spectacles or contact lenses.

A possible measure of success of CXL should be the proportion of treated eyes/subjects that progress to corneal transplantation, compared to eyes/subjects that do not undergo CXL. However, since CXL cannot be ethically withheld in cases of progressive keratoconus, the rates of progression to corneal transplantation in subjects with/without CXL can no longer directly be investigated. Nonetheless, even if the effectiveness of CXL is reduced in subjects/eyes with greater disease severity, there is a clear benefit in its implementation, in terms of patient morbidity relative to corneal transplantation and cost effectiveness.

Assessment of the lifetime cost of treating keratoconus, including costs of clinic visits, fitting fees for contact lenses, the contact lenses themselves, surgical procedures, and complications, is estimated to be USD$25,168 greater than that of treating myopia. More than half of the increased cost of treating keratoconus (USD$13,994) is attributed to the cost of performing a penetrating keratoplasty (PKP) and dealing with post-operative complications.

It is therefore apparent that the use of CXL should provide a significant cost saving measure in keratoconus as it has been shown to reduce the corneal transplantation rate for keratoconus. Indeed, the cost of performing CXL, including the necessary pre-operative and post-operative care and resources, has been estimated to be considerably lower than that of a PKP at USD$1,929. Of note, the estimate of USD$1,929 was produced for an optometrist conducting the CXL procedure and
associated clinic visits with an expected increase in cost of 20% if the same services were delivered by an ophthalmologist. However, the cost estimate was produced for the Dresden protocol and utilising an A-CXL protocol with a shorter UV-A exposure time would cost even less as a large contributor to the overall cost of CXL is the time taken to conduct the procedure, estimated to be approximately USD$600 to pay the optometrists wages with the procedure taking 105 minutes to complete.

As indicated in chapter 6 (ARK Part IIB) individuals with keratoconus in NZ have not traditionally been followed up in tertiary care, as once the diagnosis is made they generally remain under the care of their optometrist for refractive correction. However, with the paradigm shift toward CXL becoming standard of care for progressive keratoconus, individuals with the condition will need to be monitored more closely for progression. It is likely that the majority of monitoring of individuals with keratoconus for progression will occur in tertiary care in NZ as this is where the appropriate equipment required to carry out this task is currently located. Indeed, it appears that only approximately 1/3 optometry practices in NZ have access to a computerised corneal topographer, furthermore these are generally Placido ring based devices and only 43.5% of practices have access to a pachymeter which is likely to be of the ultrasound variety. Hence, the majority of optometry practices in NZ do not have the equipment necessary to accurately assess the progression of keratoconus. Nonetheless, as indicated by ARK Part I, approximately 2000 patients with keratoconus are currently under the care of optometrists in NZ, and although the referring optometrists may represent a large section of those interested in keratoconus management, the number of cases is likely to be a significant underestimate. Therefore, the primary care optometry setting may be ideal for monitoring individuals with keratoconus for progression but only if technical resources e.g. tomographers, are widely upgraded.

In general, the results of ARK Part I are useful in informing resource allocation decisions by government and District Health Boards in NZ. Indeed, while only 10.5% of optometrists were surveyed in ARK Part I, some practices provided data on 100-400 participants. Thus it may be cost effective to target these practices, providing an appropriate computerised corneal tomographer and outsourcing the monitoring of individuals with keratoconus with a referral to tertiary care for CXL only once progression is confirmed, thus easing the already excessive burden on tertiary care. Moreover, ARK Part I assists in forming an evidence base from which planning decisions can be made regarding present and future government funding of the appropriate interventions to best manage keratoconus in NZ including; contact lenses, CXL and corneal transplantation.
Another application of the results of ARK Part I is to provide the Royal Australian and New Zealand College of Ophthalmologists (RANZCO) and New Zealand Association of Optometrists (NZAO) with indications of the appropriate numbers of ophthalmologists and optometrists required to treat keratoconus. This might enable appropriate planning to ensure adequate numbers are trained to appropriately manage individuals with keratoconus in terms of monitoring for progression, treating with CXL and corneal transplantation, and fitting contact lenses.

11.3 Conclusions

The ARK Study: Part I has improved our understanding of the epidemiology of keratoconus in NZ, possibly confirming the long held clinical suspicion that keratoconus has an ethnic predisposition for individuals of Māori and Pacific Peoples descent. However, a deficit in our understanding of the epidemiology of keratoconus in NZ persists as ARK Part I was not able to produce a meaningful estimate of prevalence which requires further investigation. Nonetheless, ARK Part I has significant potential to assist with resource allocation decisions to be made by the NZ government to treat keratoconus now and in the future, ensuring that there are minimal barriers to implementing contact lenses, CXL and corneal transplantation when required, and planning for the appropriate training of health professionals to manage keratoconus by RANZCO and NZAO. Additionally, ARK Part I has a role in informing the planning of methods to detect keratoconus in as many individuals as possible and at the earliest disease severity possible, such as through targeted screening of teenagers of Māori and Pacific Peoples ethnicities, to ensure CXL has the greatest chance to prevent disease progression.

The ARK Studies Part IIA, B and C, have contributed to the characterisation of the natural history and phenotypic characteristics of keratoconus in NZ. ARK Part IIA, indicated that keratoconus may have a greater inter-eye disease asymmetry in NZ and that corneal tomographic features are correlated with corneal microstructure and biomechanical integrity. ARK Part IIB and C suggest that keratoconus may continue to progress beyond age 30 years and ARK Part IIB characterised the relationship between corneal curvature/power and contact lens corrected visual acuity. Nonetheless, further investigations into the natural history and phenotypic characteristics of keratoconus in NZ and around the world are still necessary to further develop our understanding of the disease risk profile and determine individual and eye specific risk factors of individuals with keratoconus that may require CXL. The paradigm shift toward CXL becoming standard of care for progressive keratoconus suggests that future investigations of the natural history of keratoconus may occur predominantly through short term prospective longitudinal or cross-sectional investigations but investigations with longer follow-up will likely be limited to longitudinal retrospective investigations.
The ARK Study Part IIIA revealed that for keratoconic eyes, simulated keratometry was most repeatable on the Pentacam HR while corneal pachymetry was most repeatable on the Galilei G2, and the Orbscan II was least repeatable in all parameters. ARK Part IIIA reiterates the importance of determining the repeatability of corneal computerised tomography devices in eyes with keratoconus in order to develop evidence based criteria for the progression of keratoconus that is specific to the device being utilised, making investigations of the efficacy of CXL more comparable.

ARK Part IIIB demonstrated that both the continuous and pulsed high irradiance, high intensity accelerated CXL protocols are safe and effective at slowing or halting disease progression at 12 months post-operatively. However, ARK Part IIIB highlights that accelerated CXL is still a relatively recent development and may require refining to obtain the optimum safety and efficacy of the procedure.

My three and a half year journey through the evolution of these wide-ranging ARK studies leaves me with the following positive views: A) a concerted effort to further refine the risk profile for the progression of keratoconus, B) the facilities to detect the condition in as many individuals at the earliest disease stage possible, C) the resources to treat with CXL those that require and are suitable for such treatment, and it is D) entirely possible that we may see a dramatic reduction in corneal transplantations for keratoconus internationally and in New Zealand/Aotearoa in the next decade. Thus I hope and envisage that it is entirely conceivable that the burden of keratoconus, both in terms of individual disability and economic impact, can be substantially reduced in the coming years.
Appendix A – Status of Publications Related to this Thesis

The contents of the following chapters have been accepted or submitted for publication in peer-reviewed scientific journals as specified. For the chapters that have been accepted and published by the journal, the article itself has been included.

Chapter 2 - Introduction to Keratoconus: How did we get to where we are? Dr John Nottingham's 1854 landmark treatise on conical cornea considered in context of current knowledge of keratoconus
- Published in the journal “Cornea” in May 2016

Chapter 4 - The Epidemiology, Demographics, Distribution and Basic Clinical Characteristics of Subjects with Keratoconus in New Zealand – The Aotearoa Research into Keratoconus (ARK) Study: Part I
- Submitted to the British Journal of Ophthalmology in July 2016 – currently under peer-review
- Title of submission: The Aotearoa Research into Keratoconus (ARK) Study: Part I – Epidemiology, Demographics and Clinical Characteristics of Keratoconus in New Zealand

Chapter 7 - The Natural History of Corneal Topographic Progression of Keratoconus after Age 30 years in Non-contact Lens Wearers - The Aotearoa Research into Keratoconus (ARK) Study: Part IIIC
- Published in the British Journal of Ophthalmology in June 2017

Chapter 8 - Repeatability and Agreement of Orbscan II, Pentacam HR and Galilei Tomography Systems in Corneas with Keratoconus - The Aotearoa Research into Keratoconus (ARK) Study: Part IIIA
- Published in the American Journal of Ophthalmology in March 2017
Appendix B – Published Articles Related to this Thesis


Dr John Nottingham’s 1854 Landmark Treatise on Conical Cornea Considered in the Context of the Current Knowledge of Keratoconus

Akilesh Gokul, BOptom, Dipika V. Patel, PhD, MRCPeth, and Charles N. J. McGhee, DSc, FRCPeth

Abstract: John Nottingham has been widely credited with the first accurate description of keratoconus in his treatise on conical cornea, published in 1854. Contained within the 270-page treatise are accounts and theories of keratoconus postulated by authors such as Scarp, von Carion, von Ammon, and Mackenzie, synthesized by Nottingham in a treatise containing his own original observations. Nottingham’s work delves deeply into keratoconus, with coverage reminiscent of a modern review, albeit in a far less succinct manner. He extensively describes the epidemiology, clinical presentation, underlying cause, and treatment of keratoconus. However, the concepts put forth are limited largely by the contemporary lack of understanding of the underlying anatomy and physiology of the eye, and the observations, by technological limitations. He postulates a similar treatment algorithm to that used today; optical devices being the management option of choice in the mild stages with surgery being a last resort. None of the surgical methods discussed are used in the modern era, but he does make reference to the possible efficacy of corneal transplantation. Nottingham’s treatise was published over 160 years ago, yet his ideas and observations are surprisingly accurate. It is very possible that he was the first person to publish an accurate, comprehensive description of keratoconus.

Key Words: keratoconus, cornea, history, John Nottingham

(Cornea 2016;35:673 678)

It is frequently stated that the first published, detailed, and accurate description of keratoconus was provided by Nottingham in 1854. He produced a 270-page treatise, consisting solely of text, with no figures or clinical drawings, entitled “Practical Observations on Conical Cornea and on the Short Sight and Other Defects of Vision Connected with it,” which was published in London, England, by John Churchill (Fig. 1). The treatise consolidated the observations of others, much like a modern-day review article; an account of the works on keratoconus before Nottingham, their context and historic precedence, has been described in a recent review. All the observations described by Nottingham were from examinations by naked eye or using a magnifying glass. Whereas Nottingham’s treatise contains no illustrations, his contemporary Friedrich August von Ammon, whom Nottingham referenced several times, published an article in 1841 containing illustrations of “cornea conica” (Fig. 2). Since the publication of Nottingham’s treatise, our view of keratoconus, in terms of pathogenesis, diagnosis, and treatment, has seen many a paradigm shift. This review analyzes Nottingham’s treatise in terms of its originality at the time of publication and compares the observations and ideas considered with those held by experts of today.

NOTTINGHAM: THE SURGEON AND HIS TIMES

Born in Yorkshire, United Kingdom, in 1810, John Nottingham studied medicine and surgery at Guy’s Hospital, London, United Kingdom, and in Paris, France, under Guillaume Dupuytren and Alfred-Armand-Louis-Marie Velpeau, followed by appointment as a house surgeon in 1837 at what was then the Liverpool Infirmary, Liverpool, United Kingdom, and undertook general practice in central Liverpool, United Kingdom, in 1840. Nottingham co-founded the St Anne’s Dispensary, Liverpool, England, where he refined his skill in ophthalmology and optometry. He published on subjects in the fields of ophthalmology and optometry. Nottingham was awarded Membership in the Royal College of Surgeons in 1832, Membership in the Royal College of Physicians, London, in 1844, and Fellowship in the Royal College of Surgeons in 1846. At the time of publication of his treatise on the conical cornea, Nottingham was a surgeon at the Southern Hospital, Liverpool, where he was appointed in 1850.

John Nottingham was an avid reader and accomplished linguist with a large personal literary and scientific library, which made him an impressive conversationist. In addition, he was a Fellow of the Royal Astronomical Society. After his retirement, he suffered from bilateral cataract until operated on in 1880 and 1881 but later had 1 eye “extirpated” because of “exposure” and acute inflammation in the winter of 1887. It is reported that he was made invalid in his final 20 years by blindness, bronchitis, and later “senile decay.” John Nottingham died at the age of 84 on May 7, 1895.
PRACTICAL OBSERVATIONS
ON
CONICAL CORNEA,
AND ON THE
SHORT SIGHT,
AND
OTHER DEFECTS OF VISION CONNECTED WITH IT.

BY
J. NOTTINGHAM, M.D.,
FELLOW OF THE ROYAL COLLEGE OF PHYSICIANS OF ENGLAND, CORRESPONDING
MEMBER OF THE MEDICAL SOCIETY OF EDINBURGH, OF THE SOCIETY
MEDICAL SOCIETY OF LIVERPOOL, OF THE ACADEMY OF MEDICINE AND
ACADEMY OF MEDICINE AND
Academy of Natural Sciences of Paris; Surgeon to
the St. Ann’s Eye and Ear Institution,
LIVERPOOL.

LONDON:
JOHN CHURCHILL.
LIVERPOOL: DEIGHTON & LAUGHTON.
1854.

FIGURE 1. Title page of John Nottingham’s treatise: “Practical Observations on Conical Cornea and on the Short Sight and Other Defects of Vision Connected with it.”
FIGURE 2. Illustrations of "cornea conica" by Nottingham's contemporary, Friedrich August von Ammon, published in 1841.

EPIDEMIOLOGY

Nottingham believed keratoconus to be fairly uncommon, with an estimated prevalence below 100 and up to 300 per 100,000. Currently, the prevalence of keratoconus is estimated to range from 6.8 to 2500 per 100,000. Discrepancies in the estimates of the prevalence of keratoconus may be because of numerous factors, namely, the diagnostic criteria used, the geographic location where the study was conducted, and the fact that most prevalence studies of keratoconus are case based.

Nottingham suggested the idea of ethnic predilection, stating that conical cornea had been observed and treated in Mongolians and whites with a preference for the Mongolian (Chinese) people. It has been observed and treated of as occurring in the Mongolian and white branches of the human race, and in greater proportion in the former, as observations made in China tend to show." Nottingham also quotes a report by Sir John Richardson, stating he "observed no cases of conical cornea among the inhabitants of the northern regions of America" including among the native "Esquimaux" and "Indians." Modern epidemiological studies have confirmed ethnic variations in the incidence of keratoconus and extended these observations to other ethnicities. For example, the prevalence of keratoconus is greater in India (2.3%) and Israel (2.34%) than in Denmark (0.086%).

In discussing the epidemiology of keratoconus, Nottingham noted the limitation of clear delineation of the disease when making a diagnosis, observing that many cases of keratoconus would remain undetected as patients would consider it to be "weakness of the eye" and present to an "optician instead of a surgeon." In contrast, most modern epidemiological studies of keratoconus occur in tertiary centers such as hospitals, where generally more advanced cases of keratoconus are managed. Thus, these patients probably constitute the minority of cases in any community. Indeed, advances in rigid gas-permeable lens technologies over the past 30 years have made it possible for most cases of mild to moderate keratoconus to be managed by community optometrists.

CLINICAL SIGNS

The prevailing method of assessing the cornea and anterior segment of the eye, used by Nottingham and other observers in the same period, was by means of the naked eye with or without a simple magnifying lens. Remarkably, with these limited resources, Nottingham was able to describe the conical protrusion, eccentric apex, and corneal thinning that occur in keratoconus. He also noted that the cornea adopted a "sparkling brilliancy" as if "a large and fluid tear is holding on its anterior surface," the credit for which is given to the "peculiar curve of the cornea and associated luminous reflection and refraction." This may have been in part a description of what we now call Rizzuti's sign or the internal reflection observed at the apex in severe keratoconus. The subsequent invention of the slit-lamp made possible the observation of many of the clinical characteristics of keratoconus that are now considered common knowledge. Corneal tomography now allows us to analyze corneal shape in much greater detail.

Nottingham provided a detailed description of acute corneal hydrops, which he referred to as "hydrops oculi." He noted the loss of transparency and swelling of the cornea, which he correctly attributed to accumulation of the aqueous humor and even alluded to the link with trauma, which he related in a case he observed; "the thin tunic was burst by a slight blow on the eye."

ETIOLOGY AND PATHOGENESIS

Keratoconus remains an enigmatic disease in many ways despite the enormous diagnostic and technological advances over the last 160 years. Nottingham recognized that keratoconus may be the end result of different underlying mechanisms. In direct connection with this, he proclaims that keratoconus presented at ages that were too varied and circumstances so different that it would be unlikely that there was one common cause. However, his theories of the etiology of keratoconus and the nature of many of the case reports included in his treatise beg the obvious question; was Nottingham always describing keratoconus? In the context of our current knowledge of corneal disease, because other causes of thinning, scarring, and protrusion would have been difficult to discriminate in later stages with the limited diagnostic equipment available at that time, it is reasonable to speculate that Nottingham and his contemporaries included some nonkeratoconus cases in their analyses.

Nottingham made several clear inferences of keratoconus being a hereditary condition, in one instance stating that the condition affected "several children born of the same
parents, and not infrequently, several members of the same stock in successive generations.” In addition, he provided a number of arguments implicating environmental insults that may cause the disease. Nottingham1 believed the larger number of keratoconus cases in China to be because of the climate, meteorological conditions, and the habits of the Chinese, in one instance reporting that “the injurious effects of an everyday practice of the Chinese barbers, who cleanse or “wash” the eyes of the people with an ivory or bamboo instrument, sharply rubbed into the cornea, and deeply into the canthi. This “operation” leaves the eye red and irritated and may have been related to conical corneas.1

Nottingham1 also queried whether the smoke in the huts of the Chinese and Northern Europeans predisposed them to keratoconus. Since the time of Nottingham, the hereditary component of keratoconus has been well documented with diverse international studies reporting a family history of disease in 5% to 20% of patients.8,15 It has also been demonstrated that clinical keratoconus can occur in one but not the other twin in a pair of monozygotic twins, indicating that exogenous factors may indeed have a significant influence.8,17

A number of environmental factors are associated with the development of keratoconus, including atopic eye disease, such as vernal keratoconjunctivitis and allergic conjunctivitis, which are associated with more severe keratoconus.18 Though atopy is related to a person’s susceptibility, the occurrence and severity are determined by the environment. Vernal keratoconjunctivitis, for example, occurs in warm climates, where it tends to be more severe.19 Other exogenous associations of keratoconus include contact lens wear and eye rubbing.20,21 Both of which are implicated in the pathological mechanisms discussed already. Interestingly, chronic eye rubbing may not be so different in effect from the “eye washing” procedure described by Nottingham.

Among the cases discussed by Nottingham,1 many were associated with inflammation caused by a multitude of conditions ranging from trauma to smallpox and including iritis and keratitis. In fact, keratoconus is believed to be a noninflammatory condition, though recent biochemical discoveries including elevated levels of inflammatory mediators, such as tumor necrosis factor α and multiple interleukin molecules, including interleukin 6 and interleukin 17,20,23 have challenged this view. Nonetheless, despite the implication of low-level inflammation in the pathogenesis of keratoconus, the cardinal clinical signs of inflammation are typically absent with the exception of acute corneal hydrops.

Nottingham also spoke of alterations in the nutrition of the cornea in keratoconus. Of particular note are his statements with regard to the 2 different “modes of origin”: “The first class of cases may be spoken of as instances of conical cornea from alteration in the nutrition of this membrane without visible traces of inflammatory action” and “the second class, as cases of conical cornea, also from perverted nutrition, but preceded or accompanied, or both, by the visible phenomena of inflammation.”24 We now understand that keratoconus may be the result of alterations to a number of corneal homeostatic processes. These include increased keratocyte apoptosis,24 enzymatic degradation,25,26 oxidative stress,27,28 and collagen lamellar slippage.29,30

Nottingham also proposed that anomalies of the nervous system contributed to the formation of the conical cornea noting “the manner in which the nutrition of the cornea may, fairly be supposed to be perturbed, in connection with the which the thought suggests itself, that some altered condition of innervation might here be taken into account.” Modern studies using in vivo confocal microscopy have confirmed that the microstructure of the corneal subbasal nerve plexus, but has been shown that the normal pattern of radiating spokes converging to a whorl-like region29 is lost in keratoconus. Instead, the subbasal nerves form a tortuous network with closed loops and nerve bundles conforming to the base of the cone in a concentric pattern.30

**TREATMENT**

Nottingham characterized 4 relatively broad categories for the treatment of keratoconus, practiced by him and others: Optical, Medical, Surgical, and Mechanical. He noted the variability in success of these treatment modalities, “It is worthy of remark, that scarcely any of 2 practices have been equally fortunate with the same remedial means,” indicating the difficulty with which keratoconus was treated.

Nottingham1 stated that “optical instruments, in the earlier stages, or the case may be deferred to be operated on, may be of great use.” He prescribed “biconvex” lenses, in many instances with reported success. He also alluded to the use of what may be a form of Galilean telescope, used for improving vision: “Combinations of lenses of different figures, the ocular one biconvex, the distal convex, with adjusting apparatus.” Finally, he described the optical efficacy of pin-hole goggles in keratoconus and also alluded to the potential use of corneo-scleral shells, stating “Lenses with posterior surface corresponding to the front of the eye, and anteriorly of regular figure, are amongst the means proposed for correcting the altered refraction; along with these may be mentioned lenses of transparent animal jelly, contained in capsules of glass, to be placed on the front of the eye.”30 Of all the optical management options discussed by Nottingham, none truly address the major underlying cause of vision loss in keratoconus, that of an irregular corneal shape. It was not until the late 19th century that glass corneo-scleral shells were fully developed, and with improvements in design and material technology, these subsequently evolved into the modern range of contact lenses.

Nottingham mentions a contemporary range of medical treatments for keratoconus, including oral sulfate of zinc in combination with sulfate of magnesia, arsenic, tincture of sesquichloride of iron with aloe, and myrrh. Topical treatment with nitric oxide of mercury ointment was also reported. In modern medicine, no such remedies are used as they have never been proven to treat keratoconus. Indeed, to date there have been no useful advances in medical treatments for keratoconus per se. However, recent research as
previously noted, by identifying molecular components that are implicated in keratoconus, may provide potential targets for future therapeutic interventions.

Nottingham’s treatise discusses a great many surgical procedures for the treatment of keratoconus, performed by him and others. However, he was reluctant to advocate any surgical procedure, considering their limited success rate, and he advocates their use only when all other options have been exhausted; stating: “It is obvious that, before proceeding to surgical operation, the different forms of optical contrivance should be resorted to, and their various combinations carefully tried when the more simple ones fail to assist vision.” Surgical interventions in the mid-19th century were limited by surgical instrumentation and technology, a lack of therapeutic agents such as antibiotics and steroids, and limited knowledge of the anatomy and physiology of the eye. However, some of the surgical procedures discussed illustrate a reasonably accurate understanding of keratoconus. Surgical techniques described include anterior chamber paracentesis to reduce intraocular pressure, thereby flattening the cornea, creation of an eccentric artificial pupil to improve visual function, corneal or excision of the conical part of the cornea, division of the recti muscles to alter abnormal action on the cornea, and removal of part of the cornea to promote scarring and corneal flattening, all of which met with little success and do not have comparable procedures in modern times. Corneal transplantation is mentioned in passing as a potentially effective surgical intervention, whereas in the modern day, corneal transplantation for keratoconus is a mainstay of treatment and generally has better visual outcomes and survival rates than other indications.

Patients now benefit from a host of treatment options aimed at restoring vision and slowing disease progression, many of which are the result of a better understanding of the natural history of the condition. These include corneal collagen cross-linking, insertion of intraconal ring segments or intraocular lenses, and corneal transplantation. Along with the much improved repertoire of treatment options, consensus regarding definitions of diagnosis, progression, and management of keratoconus is likely to see improvements in patient outcomes in the years to come.

CONCLUSIONS

In an age of technological wonder and research, we must not forget the foundations laid by our predecessors that have allowed us to reach such an intricate understanding of diseases such as keratoconus. John Nottingham was the first to publish a vivid and comprehensive description of the condition. Given the technological limitations of the time, many of his observations were surprisingly accurate and many of his observations were later accepted, during his career and retirement, and thus came to form the building blocks of our knowledge of keratoconus in the second half of the 20th century.

REFERENCES


Copyright © 2016 Wolters Kluwer Health, Inc. All rights reserved.
The natural history of corneal topographic progression of keratoconus after age 30 years in non-contact lens wearers

Akilesh Gokul, Dipika V Patel, Grant A Watters, Charles N J McGhee

ABSTRACT

Background/aims: To determine if significant progression of disease occurs in older, non-contact lens wearing, subjects with keratoconus and to identify potential predictive factors.

Methods: Clinical and computerised corneal topography records of subjects with keratoconus attending a specialist optometry practice were retrospectively analysed to identify those aged ≥30 years, with ≥2 consultations ≥12 months apart, no contact lens wear and no corneal scarring, surgery or corneal hydrops. Topographic parameters assessed included: maximum keratometry (Kmax), steep keratometry (Kstee), flat keratometry (Kflat), inferior-superior (I-S) ratio and the surface asymmetry and regularity (surface asymmetry index and surface regularity index) indices.

Results: Of the 449 subjects with keratoconus assessed, 43 eyes of 27 patients (6.01%) met inclusion criteria, with median age 38.45 (12.86) years at baseline and median follow-up 4.36 (8.68) years. There was a significant increase in Kmax (0.30 (1.21) D), Kstee (0.27 (0.90) D), Kflat (0.34 (1.12) D) and I-S (0.26 (0.82) D) between baseline and final review, p<0.05. Notably, 18.6%–25.6% of eyes demonstrated ≥1.00 D increase in one or more four principal topographic parameters (Kmax, Kstee, Kflat, I-S ratio), while 18.5%–37.0% of subjects had ≥1.00 D increase in the aforementioned parameters in at least one eye over the study period. However, <10% of eyes exhibited ≥1.00 D increase/year in all topographic parameters. The only significant predictor of progression was follow-up time.

Conclusions: This study confirms that keratoconus may continue to progress beyond age 30. Older subjects with keratoconus should be monitored for progression, particularly with respect to possible corneal collagen cross-linking or astigmatic correction in cataract surgery.

INTRODUCTION

Keratoconus is an ectatic, thinning disorder in which the cornea takes up a conical shape with the increased curvature inducing irregular astigmatism, resulting in visual impairment. Typically, as keratoconus progresses the corneal curvature increases. Traditionally, corneal curvature is measured using manual keratometers; however, these devices use just four measurements localised to the paracentral cornea and have largely been superseded by computerised corneal topography/topography devices, which use several thousand data points over much of the corneal surface, providing a more detailed assessment.

The traditional view of the natural history of keratoconus is onset in early teen age years with progression up until the age of 30 or 40 years when it generally arrests. However, the full natural history of keratoconus is yet to be determined definitively, particularly beyond age 30. Previous studies have generally involved participants with a wide age range and included many subjects wearing rigid contact lenses (CLs), the latter compromising accurate, longitudinal measurement of corneal shape. Keratoconus is a relatively common disease affecting 8.6–2340/100 000 of the population; therefore, there is a considerable number of middle-aged and older subjects with mild-to-moderate keratoconus currently managed with spectacles or CLs. Better knowledge of the risk of significant disease progression in this age group would be beneficial for both those affected and healthcare providers, in terms of planning follow-up, prognosis and surgical management, including the use of collagen cross-linking (CXL).

The aims of this study were to determine (1) if significant progression of manifest keratoconus occurs beyond age 30 in non-CL wearers, based on longitudinal changes in topographic parameters and (2) if topographic disease severity and subject-specific parameters can be used to predict the likelihood of progression.

SUBJECTS AND METHODS

Patient selection

In conjunction with the Department of Ophthalmology, University of Auckland, a retrospective review was performed of the clinical records of subjects with keratoconus who underwent consultation between 2002 and 2015 at a subspecialty optometry practice with more than 60 years of specialised provision of eye care for keratoconus (Mountier Frist, Auckland, New Zealand).

The investigation was focused on patients with manifest keratoconus, thus all subjects diagnosed with keratoconus, on the basis of examination by an experienced clinician using a combination of computerised corneal topography, slit-lamp biomicroscopy and refraction, were identified (N=449). The following inclusion criteria were applied: confirmed diagnosis of manifest keratoconus, age ≥30 years and at least two consultations ≥12 months apart. Exclusion criteria included former fruste keratoconus, any previous CL wear, a history of hydrops corneae, corneal scarring, previous ocular surgery or other ocular pathology.

Clinical science

Main outcome measures
The following corneal topographic parameters and keratoconus indices were obtained from Medmont-E300 (Medmont, Camberwell, Australia, Medmont studio V6.1.1.4—all captured exams were analysed with this version), Placido ring-based axial power maps, for the first (baseline) and most recent (final) reviews for all eyes that met the inclusion/exclusion criteria; apical keratometry (Kmax), steep simulated keratometry (Ksteep), flat simulated keratometry (Kflat), inferior-superior dioptric asymmetry (i-s), surface asymmetry index (SAI) and surface regularity index (SRI). To determine the repeatability of the parameters obtained from the Medmont-E300 in patients with keratoconus, three consecutive scans were carried out on 20 randomly selected patients with keratoconus who met the inclusion criteria of the study. However, CI. wearers were included if no CI. was worn for ≥2 days. The median and IQR, within subject SD (Sw), precision (1.96 Sw) and repeatability (2.77 Sw) was calculated for all parameters.

Statistical analysis
The Kolmogorov-Smirnov test revealed that all study parameters were non-parametric. The difference for all outcome measures between baseline and final reviews, for all eyes, were compared using the Wilcoxon signed-rank test (Table 1A). The Mann-Whitney U test was used to investigate differences in all outcome measures between the repeatability and main study groups to assess differences in disease severity. Eyes were subsequently grouped according to degree of progression based on the repeatability of the topographic parameters (Table 1B); no progression (< 1.00 D change) and significant progression (≥1.00 D increase). The rate of change in the variables that demonstrated statistically significant differences between baseline and final review was assessed and the number of eyes with significant rates of progression was determined, defined using similar criteria to progression; no progression (< 1.00 D change/year) and significant rate of progression (≥1.00 D increase/year). Univariate associations between potential predictors and changes in outcome measures were evaluated; Spearman’s correlation test for continuous predictors and outcome measures and Mann-Whitney U test for dichotomous predictors; gender, age at baseline and follow-up time. The Spearman’s correlation test was also used to determine if the changes in outcome measures were correlated with each other.

Factors associated with an increase of ≥1.00 D in at least one eye for all outcome measures were performed on a subject-specific basis, using the between-eye mean of eye-specific predictors, such as Kmax at baseline, in the analyses. The initial analyses included the Mann-Whitney U test for continuous predictors and the χ² test for dichotomous predictors. All variables that were significant were then included in a logistic regression analysis to produce ORs and 95% CIs. Age at baseline and follow-up time were assessed as continuous and dichotomous predictors; age ≥40 and ≤40 years and follow-up time <5 and ≥5 years. A p value < 0.05 was considered to be significant throughout.

RESULTS
Forty-three eyes of 27 subjects met the study criteria and were included. The median age at baseline was 38.45 (12.86) years, 13 (48.1%) subjects were aged ≥40 years and 14 subjects (52.0%) were male. The median age at final review was 43.21 (14.86) years. The median follow-up time was 4.36 (6.86) years with 11 (40.7%) subjects having ≥5 years follow-up.

All topographic parameters demonstrated a significant median increase between baseline and final review (p < 0.05), except for SAI and SRI, which did not change significantly (p > 0.600) (Table 1). The repeatability analysis of the topographic parameters was conducted on 37 eyes of 20 patients and suggests that a change of ≥1.00 D is indicative of significant disease progression for all parameters except for SRI (Table 1). Kmax, Ksteep and SRI were significantly higher in the repeatability cohort.

The overall median rate of change (Δ/year) was 0.06 (0.28), 0.04 (0.19), 0.08 (0.11) and 0.04 (0.20), for Kmax, Ksteep, Kflat and i-s, respectively. The majority of eyes (93.0%–100.0%) exhibited no progression (< 1.00 D change/year) for all topographic parameters (figure 1A). A significant rate of change

Table 1 (A) Change in corneal topographic parameters between baseline and final reviews in the main study group, 47 eyes of 27 subjects; apical keratometry (Kmax), steep simulated keratometry (Ksteep), flat simulated keratometry (Kflat), inferior-superior dioptric asymmetry (i-s), surface asymmetry index (SAI) and surface regularity index (SRI). All values are median (IQR). (B) Repeatability analysis of Kmax, Ksteep, Kflat, i-s, SAI and SRI on 37 eyes of 20 randomly selected patients with keratoconus to determine criterion for progression

<table>
<thead>
<tr>
<th>(A) Variable</th>
<th>Baseline</th>
<th>Final</th>
<th>Change in parameter (final and baseline)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kmax (D)</td>
<td>50.91 (8.01)</td>
<td>52.02 (8.04)</td>
<td>0.30 (1.21)</td>
<td>0.039*</td>
</tr>
<tr>
<td>Ksteep (D)</td>
<td>46.64 (7.84)</td>
<td>47.36 (7.86)</td>
<td>0.72 (0.90)</td>
<td>0.039*</td>
</tr>
<tr>
<td>Kflat (D)</td>
<td>44.66 (5.92)</td>
<td>44.96 (5.39)</td>
<td>0.34 (1.21)</td>
<td>0.005*</td>
</tr>
<tr>
<td>i-s (D)</td>
<td>2.94 (0.68)</td>
<td>3.50 (3.74)</td>
<td>0.26 (0.62)</td>
<td>0.005*</td>
</tr>
<tr>
<td>SAI</td>
<td>3.70 (4.89)</td>
<td>4.14 (4.64)</td>
<td>-0.09 (0.88)</td>
<td>0.628</td>
</tr>
<tr>
<td>SRI</td>
<td>1.16 (0.87)</td>
<td>1.13 (0.87)</td>
<td>-0.01 (0.19)</td>
<td>0.744</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(B) Variable</th>
<th>Median (IQR)</th>
<th>Within subject SD</th>
<th>Precision</th>
<th>Repeatability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kmax (D)</td>
<td>54.59 (3.98)</td>
<td>0.33</td>
<td>0.65</td>
<td>0.92</td>
</tr>
<tr>
<td>Ksteep (D)</td>
<td>51.61 (4.95)</td>
<td>0.33</td>
<td>0.64</td>
<td>0.91</td>
</tr>
<tr>
<td>Kflat (D)</td>
<td>46.43 (5.23)</td>
<td>0.34</td>
<td>0.66</td>
<td>0.94</td>
</tr>
<tr>
<td>i-s (D)</td>
<td>3.22 (0.69)</td>
<td>0.36</td>
<td>0.71</td>
<td>1.01</td>
</tr>
<tr>
<td>SAI</td>
<td>4.22 (4.21)</td>
<td>0.38</td>
<td>0.74</td>
<td>1.05</td>
</tr>
<tr>
<td>SRI</td>
<td>1.58 (0.32)</td>
<td>0.09</td>
<td>0.17</td>
<td>0.24</td>
</tr>
</tbody>
</table>

*Statistically significant difference between baseline and final visit.
†Statistically significant difference between parameter in repeatability group and main study group.
Table 2  Spearman’s correlation test and univariate associations for change in computerised corneal topographic parameters between baseline and final review, patient factors and follow-up time

<table>
<thead>
<tr>
<th>Variable</th>
<th>Change in parameter (Δ)</th>
<th>Kmax</th>
<th>Ksteep</th>
<th>KFlat</th>
<th>I-S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at baseline review (years)</td>
<td>n=0.087</td>
<td>n=0.073</td>
<td>n=0.155</td>
<td>n=0.103</td>
<td>n=0.067</td>
</tr>
<tr>
<td></td>
<td>p=0.581</td>
<td>p=0.753</td>
<td>p=0.511</td>
<td>p=0.511</td>
<td>p=0.670</td>
</tr>
<tr>
<td>Age category at baseline review (years)</td>
<td>n=0.40</td>
<td>n=0.40</td>
<td>n=0.40</td>
<td>n=0.40</td>
<td>n=0.40</td>
</tr>
<tr>
<td>&lt;40</td>
<td>0.22 (0.41)</td>
<td>0.16 (0.14)</td>
<td>0.70 (1.15)</td>
<td>0.15 (0.60)</td>
<td>0.39 (0.99)</td>
</tr>
<tr>
<td></td>
<td>p=0.500</td>
<td>p=0.000</td>
<td>p=0.000</td>
<td>p=0.000</td>
<td>p=0.392</td>
</tr>
<tr>
<td>≥40</td>
<td>0.32 (0.55)</td>
<td>0.29 (0.21)</td>
<td>0.41 (0.86)</td>
<td>0.32 (0.99)</td>
<td>0.00 (0.00)</td>
</tr>
<tr>
<td></td>
<td>p=0.005</td>
<td>p=0.005</td>
<td>p=0.005</td>
<td>p=0.005</td>
<td>p=0.005</td>
</tr>
<tr>
<td>Follow-up time (years)</td>
<td>n=0.015</td>
<td>n=0.039</td>
<td>n=0.102</td>
<td>n=0.118</td>
<td>n=0.453</td>
</tr>
<tr>
<td></td>
<td>p=0.925</td>
<td>p=0.804</td>
<td>p=0.515</td>
<td>p=0.453</td>
<td>p=0.453</td>
</tr>
<tr>
<td>Follow time category (years)</td>
<td>&lt;5</td>
<td>0.30 (0.37)</td>
<td>-0.02 (0.51)</td>
<td>0.16 (0.84)</td>
<td>0.09 (0.94)</td>
</tr>
<tr>
<td></td>
<td>p=0.007</td>
<td>p=0.007</td>
<td>p=0.007</td>
<td>p=0.007</td>
<td>p=0.007</td>
</tr>
<tr>
<td></td>
<td>≥5</td>
<td>0.35 (0.76)</td>
<td>1.22 (1.66)</td>
<td>0.94 (1.11)</td>
<td>0.43 (1.90)</td>
</tr>
<tr>
<td></td>
<td>p=0.752</td>
<td>p=0.026*</td>
<td>p=0.017*</td>
<td>p=0.089</td>
<td>p=0.089</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>n=0.071</td>
<td>n=0.02 (0.89)</td>
<td>0.35 (0.88)</td>
<td>0.18 (1.21)</td>
</tr>
<tr>
<td></td>
<td>p=0.007</td>
<td>p=0.007</td>
<td>p=0.007</td>
<td>p=0.007</td>
<td>p=0.007</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.60 (1.28)</td>
<td>1.17 (0.61)</td>
<td>0.74 (1.32)</td>
<td>0.32 (0.99)</td>
</tr>
<tr>
<td></td>
<td>p=0.004*</td>
<td>p=0.004*</td>
<td>p=0.004*</td>
<td>p=0.004*</td>
<td>p=0.004*</td>
</tr>
<tr>
<td>Kmax baseline (Δ)</td>
<td>n=0.073</td>
<td>n=0.073</td>
<td>n=0.073</td>
<td>n=0.073</td>
<td>n=0.073</td>
</tr>
<tr>
<td></td>
<td>p=0.675</td>
<td>p=0.675</td>
<td>p=0.675</td>
<td>p=0.675</td>
<td>p=0.675</td>
</tr>
<tr>
<td>Ksteep baseline (Δ)</td>
<td>n=0.073</td>
<td>n=0.073</td>
<td>n=0.073</td>
<td>n=0.073</td>
<td>n=0.073</td>
</tr>
<tr>
<td></td>
<td>p=0.707</td>
<td>p=0.707</td>
<td>p=0.707</td>
<td>p=0.707</td>
<td>p=0.707</td>
</tr>
<tr>
<td>KFlat baseline (Δ)</td>
<td>n=0.073</td>
<td>n=0.073</td>
<td>n=0.073</td>
<td>n=0.073</td>
<td>n=0.073</td>
</tr>
<tr>
<td></td>
<td>p=0.572</td>
<td>p=0.572</td>
<td>p=0.572</td>
<td>p=0.572</td>
<td>p=0.572</td>
</tr>
<tr>
<td>Change in Kmax (Δ)</td>
<td>n=0.073</td>
<td>n=0.073</td>
<td>n=0.073</td>
<td>n=0.073</td>
<td>n=0.073</td>
</tr>
<tr>
<td></td>
<td>p=0.531</td>
<td>p=0.531</td>
<td>p=0.531</td>
<td>p=0.531</td>
<td>p=0.531</td>
</tr>
<tr>
<td>Change in Ksteep (Δ)</td>
<td>n=0.073</td>
<td>n=0.073</td>
<td>n=0.073</td>
<td>n=0.073</td>
<td>n=0.073</td>
</tr>
<tr>
<td></td>
<td>p=0.531</td>
<td>p=0.531</td>
<td>p=0.531</td>
<td>p=0.531</td>
<td>p=0.531</td>
</tr>
<tr>
<td>Change in KFlat (Δ)</td>
<td>n=0.073</td>
<td>n=0.073</td>
<td>n=0.073</td>
<td>n=0.073</td>
<td>n=0.073</td>
</tr>
<tr>
<td></td>
<td>p=0.531</td>
<td>p=0.531</td>
<td>p=0.531</td>
<td>p=0.531</td>
<td>p=0.531</td>
</tr>
</tbody>
</table>

*Statistically significant result.

(≥1.00 D/year) was observed in Kmax and Ksteep, 7.0% and 2.3% of eyes, respectively. The greater proportion of eyes (74.4%—81.4%) exhibited no progression over the follow-up period, with an overall change <1.00 D between baseline and final reviews for all topographic parameters. However, a total increase of ≥1.00 D in corneal topographic parameters was observed in 18.6%—25.6% of eyes (figure 1B).

Table 2 illustrates associations between potential predictors and progression in computerised corneal topographic parameters. The change in Ksteep and KFlat demonstrate significant positive correlations with follow-up time, while Kmax and Ksteep increased significantly in female subjects. There were significant moderate-to-strong positive correlations between the changes in all variables; r=0.4, p<0.01 in all cases.

Figure 2 and table 2 highlight the relationship between Ksteep and follow-up time as a dichotomous variable. A greater proportion of eyes experienced no progression (<1.00 D increase) in the group with <5 years follow-up (88.9% vs 56.3% for <5 and ≥5 years follow-up, respectively), while a greater proportion of eyes with ≥5 years follow-up experienced significant progression (≥1.00 D increase, 11.1% vs 43.7%).

Table 3 shows the predictors for ≥1.00 D increase in a topo- graphic parameter in at least one eye. Follow-up time as a continuous variable and gender were significantly associated with Ksteep (p=0.031) and Kmax (p=0.004), respectively.

The results of the logistic regression analysis demonstrate that follow-up time is a significant predictor for ≥1.00 D increase in Ksteep in at least one eye (OR 1.38; 95% CI 1.04 to 1.84, p=0.026), while male gender was significantly associated with a reduction in the risk of ≥1.00 D increase in Kmax in at least one eye (OR 0.048, 95% CI 0.005 to 0.490, p=0.010).

DISCUSSION
The pathogenesis, mechanisms and rate of progression of keratoconus are not completely understood. It has been noted that there is an increase in the amount of non-enzymatic cross-linking with increasing age, likely due to exposure to ultraviolet radiation throughout life, which is a possible mechanism by which keratoconus progression is slowed or halted with increasing age. Additionally, the Collaborative Longitudinal Evaluation of Keratoconus (CLEK) reported that fewer patients progressed to transplantation later in life (12%–20% aged 10–40 years vs 8%–3% aged ≥40 years) and that older age at baseline was protective against requiring transplantation (OR 0.72).14

The current study assessed the progression of keratoconus using quantitative computerised corneal topography, and we believe this study is unique in that it is focused on the natural history of subjects with keratoconus: (1) that had never worn CLs, (2) assessment focused on computerised topography parameters at baseline and final review (median follow-up 4.36 (8.68) years) and (3) that were typically a little older than those in investigations of keratoconus. The lack of previous
CL wear avoided the potentially confounding effects of CL wear on corneal shape and possibly the keratoconus disease process. The majority of variables investigated increased significantly between the baseline and final reviews (Kmax 0.30 D, Ksteep 0.27 D, Kflat 0.34 D and 1-S 0.26 D), suggesting that corneal curvature in patients with keratoconus over age 30 continues to increase. Additionally, changes in each parameter had a moderate-to-strong positive correlation with the changes in all other parameters, thus an increase in one parameter may be accompanied by a similar increase in others, suggesting that all topographic parameters investigated could potentially be used to monitor progression.

The results of this study are somewhat comparable to the landmark CLEK, which prospectively tracked changes in corneal curvature over 8 years in 1032 subjects with mean age 38.9 years at enrolment. Measured using manual keratometer, the CLEK reported that Kflat increased at a mean rate of 0.18 ±0.60 D/year. However, the authors suggested that progression is non-linear with the greatest rate of progression occurring earlier in life; average rate of increase in Kflat of 1.0 D/year age <20, decreasing to approximately 0.4 D/year age 20–29, 0.2 D/year age 30–39 and 0.1 D/year age ≥40. The median rates of change in our cohort (median age 38.5 years) were between 0.06 and 0.08 D/year for all outcome measures, similar to that reported in the CLEK for patients aged ≥40 years. Notably, over 90% of eyes in our study cohort had <1.00 D change/year, thus change occurs at a slow pace. An increase of ≥3.00 D in Kflats over the duration of the CLEK was considered significant and occurred in 39.1% of patients aged <30 years, but 19.8% aged ≥30 years. The authors of the CLEK hypothesised that the natural history of keratoconus is dichotomous; the majority of progression occurring before age 35, which revealed that patients aged <35 years are more likely to have an increase of ≥3.00 D in Kflat in at least one eye (OR 3.12). Interestingly, in the current study using computerised corneal topographic measures, 21% of eyes in this older cohort demonstrated an increase in Kmax approaching 3.00 D.

The Medmont-E300 has been shown to be highly repeatable on normal corneas, however, unlike other topography instruments, it has not been verified on keratoconic corneas. The surface irregularity of keratoconic corneas has been demonstrated to reduce repeatability of topography devices. The current investigation performed a repeatability analysis on keratoconic corneas in order to produce categorical criteria for progression and found that ≥1.00 D increase in parameters indicated significant progression. A recent investigation of the natural history of corneal curvature with increasing age in normal subjects, using the Medmont-E300, found no trend towards change in corneal curvature up to age 69. Thus, utilisation of the repeatability to form criteria for progression in eyes with keratoconus is justified.

The criterion for progression (≥1.00 D increase) in this study was robust as the repeatability group had a greater average Kmax, which may reduce the repeatability of topography devices, thus, the change required for progression to be detected in the main study group was likely less than a 1.00 D increase. The current investigation elucidated that approximately 20% of eyes underwent significant progression (≥1.00 D increase in a parameter) and 80% no progression (≤1.00 D increase in a parameter), while a third of patients experienced significant
### Table 3: Factors associated with ≥1.00 D increase in at least one eye in outcome measures with significant change between baseline and final review

#### Continuous Predictors

<table>
<thead>
<tr>
<th>Variable</th>
<th>≥1.00 D increase in at least 1 eye</th>
<th>Kmax (D)</th>
<th>Ksteep (D)</th>
<th>Kfllot (D)</th>
<th>I-S (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of patients</td>
<td>Yes</td>
<td>33.3%</td>
<td>37.0%</td>
<td>37.0%</td>
<td>18.5%</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>66.6%</td>
<td>63.0%</td>
<td>63.0%</td>
<td>81.5%</td>
</tr>
<tr>
<td>Age at review (years)</td>
<td>Yes</td>
<td>39.10 (23.42)</td>
<td>37.29 (14.68)</td>
<td>36.98 (14.12)</td>
<td>34.23 (20.96)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>37.29 (12.93)</td>
<td>39.11 (10.66)</td>
<td>41.54 (14.54)</td>
<td>39.11 (12.02)</td>
</tr>
<tr>
<td>Follow-up time (years)</td>
<td>Yes</td>
<td>3.74 (1.93)</td>
<td>10.81 (7.50)</td>
<td>8.07 (8.87)</td>
<td>12.12 (9.22)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>4.37 (0.97)</td>
<td>2.70 (2.63)</td>
<td>2.89 (7.14)</td>
<td>3.32 (7.30)</td>
</tr>
<tr>
<td></td>
<td>p=0.890</td>
<td>p=0.500</td>
<td>p=0.003*</td>
<td>p=0.103</td>
<td>p=0.232</td>
</tr>
<tr>
<td>Kmax baseline (D)</td>
<td>Yes</td>
<td>53.86 (11.41)</td>
<td>52.90 (4.85)</td>
<td>52.86 (7.05)</td>
<td>52.99 (5.56)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>50.37 (5.76)</td>
<td>50.30 (8.46)</td>
<td>50.37 (7.02)</td>
<td>50.39 (7.35)</td>
</tr>
<tr>
<td></td>
<td>p=0.286</td>
<td>p=0.286</td>
<td>p=0.639</td>
<td>p=0.284</td>
<td></td>
</tr>
<tr>
<td>Ksteep baseline (D)</td>
<td>Yes</td>
<td>50.12 (12.17)</td>
<td>48.00 (3.33)</td>
<td>47.15 (7.51)</td>
<td>47.64 (9.75)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>46.30 (4.17)</td>
<td>46.82 (8.57)</td>
<td>47.64 (5.60)</td>
<td>47.15 (5.59)</td>
</tr>
<tr>
<td></td>
<td>p=0.322</td>
<td>p=0.639</td>
<td>p=0.537</td>
<td>p=0.694</td>
<td></td>
</tr>
<tr>
<td>Kfllot baseline (D)</td>
<td>Yes</td>
<td>48.89 (7.66)</td>
<td>47.15 (7.61)</td>
<td>44.69 (6.08)</td>
<td>45.54 (8.96)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>44.09 (2.76)</td>
<td>47.64 (7.34)</td>
<td>45.39 (4.94)</td>
<td>44.85 (5.95)</td>
</tr>
<tr>
<td></td>
<td>p=0.018</td>
<td>p=0.604</td>
<td>p=0.786</td>
<td>p=0.100</td>
<td></td>
</tr>
<tr>
<td>I-S baseline (D)</td>
<td>Yes</td>
<td>1.26 (0.23)</td>
<td>3.00 (0.22)</td>
<td>3.00 (0.60)</td>
<td>3.00 (0.20)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>3.09 (3.33)</td>
<td>2.89 (3.65)</td>
<td>3.00 (3.87)</td>
<td>2.89 (4.64)</td>
</tr>
<tr>
<td></td>
<td>p=0.628</td>
<td>p=0.803</td>
<td>p=0.978</td>
<td>p=0.717</td>
<td></td>
</tr>
</tbody>
</table>

#### Categorical Predictors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>≥1.00 D in at least one eye</th>
<th>≥1.00 D in at least one eye</th>
<th>≥1.00 D in at least one eye</th>
<th>≥1.00 D in at least one eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow-up time (years)</td>
<td>&lt;5</td>
<td>18.5</td>
<td>14.0</td>
<td>14.8</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>&gt;5</td>
<td>14.8</td>
<td>22.2</td>
<td>22.2</td>
<td>14.8</td>
</tr>
<tr>
<td></td>
<td>p=0.551</td>
<td>p=0.124</td>
<td>p=0.124</td>
<td>p=0.071</td>
<td>p=0.071</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>3.7</td>
<td>11.1</td>
<td>14.8</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>29.6</td>
<td>25.9</td>
<td>22.2</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>p=0.004*</td>
<td>p=0.689</td>
<td>p=0.293</td>
<td>p=0.538</td>
<td></td>
</tr>
<tr>
<td>Age category at baseline</td>
<td>≥40</td>
<td>18.5</td>
<td>22.2</td>
<td>25.3</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>&gt;40</td>
<td>14.8</td>
<td>14.8</td>
<td>11.1</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>p=0.555</td>
<td>p=0.402</td>
<td>p=0.147</td>
<td>p=0.538</td>
<td></td>
</tr>
</tbody>
</table>

*Statistically significant values are shown in bold.  
**Statistically significant result.

progression in at least one eye. While the changes in the anterior topographical parameters documented in this investigation have been verified as a means of detecting progression, recent evidence suggests that changes in the posterior surface may occur prior to the anterior surface. The Placido-based Medmont-E300 does not enable evaluation of the posterior corneal surface or corneal thickness, thereby limiting our definition of progression to changes in anterior topographical parameters.

The characteristics of subjects with keratoconus and risk factors for progression have been established by prospective observational studies such as CLEK and the Dundee University of Keratoconus Study. The CLEK in particular established that the greatest predictors for progression were younger age and greater disease severity at baseline. However, the CLEK quantified corneal shape by keratometry, not computerised corneal topography and a large number of participants were rigid CIs, which may have caused corneal distortion/warpage. In the current study, the only significant predictor of disease progression was follow-up time; therefore, it is possible that given enough time, additional subjects in the study cohort may experience significant progression. However, the large range of follow-up durations poses a limitation in estimations of rate of progression, as theoretically eyes with longer follow-up duration might have experienced progression following the baseline visit but progression had ceased before the final visit.

In the current study, male gender appears to be protective against ≥1.00 D increase in Kmax in at least one eye (OR 0.048), although this may reflect a limitation of the sample size. Additionally, all baseline topographic parameters were higher in the group with ≥1.00 D change in at least one eye at baseline, implicating greater disease severity as a potential predictor of progression, despite none of these baseline parameters being statistically significant. Once more this may be a limitation of the sample size as a number of prior studies have reported steeper keratometry to be a significant predictor of progression. The sample size in the current study was limited largely due to strict inclusion/exclusion criteria limiting it to subjects with keratoconus who had never worn CLs; generally, these subjects have mild-to-moderate rather than severe disease. Indeed, presuming that disease severity may be a predictor for progression, subjects with greater disease severity may have already progressed to CL wear or even corneal transplantation by this age, so it would be difficult/impossible to accurately assess disease progression by computerised topography. In this respect, it is notable that of 449 subjects with keratoconus assessed for this
study, 94% had worn CLs, had developed corneal scarring or had progressed to corneal transplantation and only 6% of subjects met the inclusion criteria. Interestingly, keratoconus has been the most common indication for corneal transplantation in New Zealand over the last two decades. Changes in visual acuity, spectacle refraction and the effect of commonly known, non-conical topographic associations of keratoconus including family history, eye rubbing and astigmatism could not be investigated due to variations in recorded history, the recording of spectacle prescription and the occasional provision of reduced cylindrical correction to maximise spectacle tolerance.

Overall, this study highlights that keratoconus may continue to progress in apparently clinically stable non-CL-wearing subjects beyond the fourth decade; however, <10% of eyes may progress at ≥1.00 D/year. These data suggest that subjects with keratoconus, over the age of 30 years, should still be monitored for progression long term, as on average one in three may experience significant progression in at least one eye, although this proportion might be higher in patients with more severe disease and those with longer follow-up. Indeed, 21% of eyes exhibited a mean increase of 2.73 D in Kmax over the duration of the study. This has significant implications for the consideration of CXL, particularly in older subjects with keratoconus progressing at a significant rate, as well as the use of toric intraocular lenses in the context of cataract surgery in keratoconus. However, CXL may not yield a significant benefit in patients with very slowly progressing disease.

Acknowledgements AG received support for his doctoral studies through a University of Auckland Doctoral Scholarship and a New Zealand Association of Optometrists Post Graduate Scholarship.

Contributors All the authors contributed to the design of the study. AG and GAW carried out the study, analysing patient records and collecting data. AG performed the statistical analysis of the data. All the authors contributed to writing the paper. AG produced the draft manuscript which was edited and enriched by DPH, GAW and CsIL. All authors approved the final version.

Competing interests None declared.

Ethics approval Ethical approval for the study was obtained from the University of Auckland Human Participants Ethics Committee (approval number: 010547).

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

Repeatability and Agreement of Orbscan II, Pentacam HR, and Galilei Tomography Systems in Corneas With Keratoconus

JAY J. MEYER, AKILESH GOKUL, HANS R. VELLARA, ZAK PRIME, AND CHARLES N.J. McGHEE

• PURPOSE: To assess the repeatability and agreement of keratometry and pachymetry measurements obtained using 3 tomographers in eyes with keratoconus.
• DESIGN: Reliability analysis.
• METHODS: SETTING: Institutional. STUDY POPULATION: Fifty eyes of 50 participants with keratoconus. OBSERVATIONAL PROCEDURE: Steep keratometry, flat keratometry, central corneal thickness (CCT), and thinnest corneal thickness (TCT) measurements using Galilei, Orbscan II, and Pentacam HR. MAIN OUTCOME MEASURES: Repeatability was assessed using within-subject standard deviation ($\sigma_W$), coefficient of variation (CV), and intraclass correlation coefficient (ICC). Bland-Altman plots and 95% limits of agreement (LoA) were used to evaluate agreement between device pairs.
• RESULTS: For all studied parameters, ICC was $>0.97$ with the least repeatable measurements obtained using Orbscan II. Mean steep keratometry values were similar while mean flat keratometry values were significantly different between all devices. The Galilei and Pentacam HR had the lowest 95% LoA for both CCT and TCT. There were no significant differences in mean CCT between Galilei and Pentacam HR. Mean Orbscan II CCT measurements were not significantly different overall but had wide 95% LoA with Pentacam HR (−47.95 to 58.09 μm) and Galilei (−43.70 to 53.91 μm). Mean Orbscan II CCT measurements were significantly lower when an asymmetric factor of 0.92 was applied ($-33.6 \mu m$ vs Pentacam HR, $P < .001$; $-33.6 \mu m$ vs Galilei, $P < .001$).
• CONCLUSIONS: Keratometric and pachymetric measurements of keratoconic eyes obtained by Galilei, Orbscan II, and Pentacam were disparate. Measurements were less repeatable with Orbscan II compared with Pentacam HR and Galilei, although overall repeatability was high for all instruments. (Am J Ophthalmol 2017;175: 122–128. © 2017 Elsevier Inc. All rights reserved.)

KERATOCONUS IS A DISEASE CHARACTERIZED BY steepening and thinning of the cornea. Accurate and precise measurements of the cornea are required for the diagnosis and monitoring of corneal ectatic diseases such as keratoconus. Measurements of corneal curvature and thickness are most frequently used to make the diagnosis of keratoconus and to monitor for progression of the disease. In addition, some treatment options, including corneal collagen cross-linking and intracorneal ring segments, require accurate pachymetry measurements to determine whether these procedures can be safely performed.

There are several commercially available instruments for the measurement of corneal curvature and thickness, which differ in the technology used. The Orbscan II device (Bausch & Lomb, Rochester, New York, USA) uses slit-scanning and Flacido disc technology to combine keratometry measurements with assessment of the anterior and posterior corneal surfaces, which allows a 3-dimensional reconstruction of the cornea.1 More recently available tomographers rely on the Scheimpflug principle, whereby imaging with a wide depth of focus allows a planar object that is not parallel to the image plane to be in focus.2 The Pentacam HR system (Oculus, Wetzlar, Germany) uses a single rotating Scheimpflug camera and monochromatic slit-light source in combination with a static camera to obtain multiple slit images, which correspond to specific angles along the optical axis.3 The Galilei Dual Scheimpflug Analyzer (Ziemer, Port, Switzerland) combines dual rotating Scheimpflug cameras and a Flacido disc to assess the anterior segment.2

Our center previously reported on repeatability and agreement of these 3 instruments when measuring healthy corneas.1 However, there is a paucity of data describing the performance of these instruments when measuring corneas that significantly deviate from normal parameters, for example, keratoconic corneas. The aim of this study was to assess the repeatability and agreement of keratometry and pachymetry measurements obtained using the Orbscan II, Pentacam HR, and Galilei tomographers in eyes with keratoconus.

METHODS

This prospective, comparative study enrolled subjects with keratoconus diagnosed by slit lamp and/or
tomography attending the Cornea and External Eye Disease Service, Greenlane Hospital, Auckland District Health Board, Auckland, New Zealand. Exclusion criteria included corneal scarring or edema visible on slit-lamp examination, history of ocular surgery or trauma, and contact lens wear. One eye from each subject was selected. The study was approved by the Northern A Health and Disability Ethics Committee, a branch of the Ministry of Health in New Zealand, and informed written consent was obtained from the participants.

The 3 devices evaluated were the Orbscan II, Pentacam HR, and Galilei Dual Scheimpflug Analyzer. All 3 tomographs were calibrated prior to beginning the study. No eye drops were applied prior to testing. Three measurements were taken per eye using each of the 3 machines in a random order. Subjects were instructed to blink immediately prior to each thickness measurement. Every measurement was conducted in a darkened room by 1 of 2 experienced investigators (A.G. and H.R.V.) in the University of Auckland Ocular Imaging Unit, within the aforementioned Cornea and External Disease Unit. For each subject, all measurements were performed within a 30-minute time period.

Both the Pentacam HR and Galilei provide a “quality score” of individual measurements. The Pentacam HR provides a quality specification of “OK” if the scan is of acceptable quality. The Pentacam HR system also allows the acquisition of 3-dimensional scans using a standard- or high-resolution mode. Measurements were taken using the standard-resolution mode (25 images per scan). The Galilei breaks down the percentage quality of the image into 4 components: motion compensation, placido, Scheimpflug, and motion distance. These components are then summarized as an overall quality score along with a reference minimum required percentage score. The Orbscan II does not provide a quality score but instead automatically discards measurements deemed to be of unacceptable quality. Where specifically noted, an acoustic correction factor of 0.92 was applied to Orbscan II data. Up to 6 total scans were attempted, per machine, if the quality of any of the first 3 scans was <85% by Galilei, or deemed “OK” by Pentacam HR, or deemed of poor quality on Orbscan II.

Statistical analysis was performed using SPSS 19.0 for Windows (SPSS, IBM, Chicago, Illinois, USA). Where data were demonstrated to have a normal distribution, as shown by the 1-sample Kolmogorov-Smirnov test, parametric analyses were used. Statistical P values <.05 were considered significant. The following keratometric parameters were assessed: steep and flat simulated keratometry values (also called maximum and minimum simulated keratometry on the Orbscan II display). Pachymetric parameters included the central corneal thickness (CCT) and thinnest corneal thickness (TCT). The repeatability of the total corneal densitometry score reported by the Pentacam HR was also assessed. Corneal densitometry is an objective method of assessing corneal clarity. Reduced corneal clarity or “haze” increases the amount of backscattered light, which is detected by the Pentacam HR and measured in grayscale units (GSU). GSU is a proprietary, relative scale ranging from 0 to 100, where 0 equates to minimum backscatter (highest transparency) and 100 to maximum backscatter (lowest transparency).

Intraobserver repeatability was assessed using within-subject standard deviation (SWS), precision, intraclass test-retest variability (repeatability), coefficient of variation (CV), and intraclass correlation coefficient (ICC). Precision was calculated as 1.96 times SWS, since for 95% of observations, the difference between a subject’s measurement and the true value would be expected to be less than 1.96 SWS. Test-retest variability, or repeatability, was calculated as 2.77 times SWS. The within-subject CV was calculated as the SWS divided by the overall mean and expressed as a percentage. The ICC is defined as the ratio of the between-subjects variance to the sum of the combined within-subjects and between-subjects variance. The ICC values range from 0 to 1, with 1 indicating perfect agreement.

Bland-Altman plots were used to compare measurements between device pairs by plotting the differences between measurements against their mean along with a transformation of the limits of agreement (LoA). The 95% LoA (mean difference ± 1.96 × standard deviation) define the range within which most differences between measurements from the 2 devices will lie.

RESULTS

FIFTY CONSECUTIVE EYES (25 RIGHT, 25 LEFT) OF 50 PARTICIPANTS (31 male, 19 female) were assessed and included in the analysis. The mean age was 23.33 ± 7.69 years. Eyes were not excluded based on the quality of the scans. However, 12 eyes had at least 1 scan with a quality score below 85% as measured by the Galilei and 6 eyes had at least 1 scan not deemed “OK” by Pentacam HR. Using the Pentacam HR classification of keratocous severity, 5 eyes were grade I, 6 eyes grade II–III, 11 eyes grade II, 9 eyes grade II–III, 14 eyes grade III, and 5 eyes grade IV.

- REPEATABILITY OF CORNEAL POWER MEASUREMENTS: Repeatability parameters for steep and flat simulated keratometry measurements are summarized in Table 1. The Pentacam HR showed the highest repeatability and Orbscan II the least repeatability of steep and flat simulated keratometry measurements. The ICC was greater than 0.98 for all devices.

- AGREEMENT OF CORNEAL POWER MEASUREMENTS: For the steep keratometry measurements, there were no significant differences in the mean values between instrument pairs. The mean flat keratometry values were significantly different between all instrument pairs, the highest
measurements obtained by the Galilei, followed by the Pentacam HR and then Orbscan II. Bland-Altman plots for agreement of keratometry measurements are shown in the Figure.

- **REPEATABILITY OF PACHYMETRY MEASUREMENTS:** Repeatability parameters for CCT and TCT are summarized in Table 1. The Galilei produced the lowest 
  
  \( S_p \) and \( CV \), and the highest ICC, indicating a higher degree of repeatability of CCT and TCT compared with the Pentacam HR and Orbscan II. The Orbscan II showed the lowest degree of repeatability. The ICC was 0.97 or higher for all devices.

- **AGREEMENT OF PACHYMETRY MEASUREMENTS:** A comparison of CCT and TCT values recorded by the 3 devices is summarized in Table 2. Mean CCT measurements were not significantly different between instrument pairs. Mean TCT measurements were significantly higher for Galilei compared with Pentacam HR. However, the Galilei and Pentacam had the closest 95% LoA for both CCT and TCT. When an acoustic factor (0.92) was applied to Orbscan II pachymetry values, mean CCT and TCT measurements were significantly lower than Pentacam and Galilei. Bland-Altman plots demonstrate the agreement of CCT and TCT between devices (Figure).

- **CORRELATION BETWEEN PACHYMETRY AND KERATOMETRY:** For each device, there was a significant inverse correlation between CCT and magnitude of steep simulated keratometry (Pentacam correlation coefficient -0.64, Galilei -0.67, Orbscan II -0.65; \( P < .001 \) for each) and flat keratometry (Pentacam -0.56, Galilei -0.67, Orbscan II -0.63; \( P < .001 \) for each). A similar inverse correlation was seen between TCT and magnitude of steep (Pentacam correlation coefficient -0.72, Galilei -0.69, Orbscan II -0.63; \( P < .001 \) for each) and flat keratometry (Pentacam -0.66, Galilei -0.63, Orbscan II -0.62; \( P < .001 \) for each).

- **CORNEAL DENSITOMETRY MEASUREMENTS:** Mean corneal densitometry measurements taken by Pentacam HR were 14.94 ± 1.50 GSU (range, 12.13–18.63), \( S_p \) 0.48, CV 3.61%, ICC 0.965. There were no significant correlations between densitometry values and within-subject standard deviation for the pachymetry or keratometry values measured by Pentacam HR, Galilei, or Orbscan II. There were also no significant correlations between densitometry values and differences in measurements between instrument pairs for pachymetry or keratometry.

**DISCUSSION**

AS MORE INSTRUMENTS FOR MEASURING ANTERIOR SEGMENT PARAMETERS BECOME AVAILABLE, THE COMPATIBILITY OF MEASUREMENTS OBTAINED USING NEWER AND EXISTING TECHNOLOGIES MUST BE DETERMINED FOR HEALTHY AND PATHOLOGIC EYES. BY COMPARING INSTRUMENTS, IT CAN BE DETERMINED WHETHER THERE IS SUFFICIENT AGREEMENT FOR MEASUREMENTS FROM DIFFERENT DEVICES TO BE USED INTERCHANGEABLY, OR...
FIGURE. Bland-Altman plots showing the agreement in steep and flat keratometry (K) measurements (diopters) and corneal thickness (central, CCT; thinnest, TCT) measurements (µm). Central line represents mean of the difference between the 2 devices. Dotted lines represent 95% limits of agreement.
directly compared. The repeatability of instruments must also be established to determine how much of a deviation from normal, or average, represents true pathology and the magnitude of change required to be considered progression of disease rather than deviation owing to test variance.

This study examined the repeatability and comparability of 3 widely used instruments, the Orbscan II, Pentacam HR, and Galilei corneal topography systems, in eyes with keratoconus. In comparison to the results from a previous study of healthy, nonkeratoconic corneas, there was more variability (based on CV) in keratometry and CCT measurements of keratoconic corneas. The Orbscan II demonstrated lower repeatability compared with the Pentacam HR and Galilei for all parameters investigated. The Pentacam HR showed the highest repeatability for keratometry measurements, whereas the Galilei showed the highest repeatability for pachymetry measurements.

The results from this study suggest that the keratometry measurements of the investigated devices cannot be used interchangeably. The mean flat simulated keratometry values were significantly different between all instrument pairs. In contrast, mean flat simulated keratometry values were not significantly different when these instruments were previously studied in healthy, nonectatic corneas, suggesting that image quality or ectasia may affect these measurements more than differences in hardware, software, or image acquisition between instruments. Though the mean steep simulated keratometry values were similar between the instrument pairs, the limits of agreement were wider than what would be clinically acceptable. We did not include standard keratometry as a reference for comparison because keratometry has not been established as a gold standard to measure corneal curvature in keratoconus. However, Pentacam measurements have been shown to be as repeatable as or more repeatable than manual keratometry when measuring healthy corneas.

Few previous studies have examined the repeatability of tomographic measurements in keratoconic eyes. Some of these studies reported mean keratometry values and did not specifically report on steep or flat simulated keratometry measurements. However, the steep and flat keratometry measurements are of importance when diagnosing keratoconus or monitoring for progression. In addition, some of these studies did not specify whether eyes were excluded based on the quality of the scans obtained.

The mean CCT measurements were similar between Pentacam HR and Galilei and Orbscan II. However, there were wide 95% limits of agreement (spanning over 90 μm) for Orbscan II measurements compared with Pentacam HR and Galilei. This indicates that, for some eyes, the CCT and TCT measurements taken by Orbscan II showed very poor agreement with Galilei and Pentacam HR. There was a trend of underestimation of CCT and TCT by Orbscan II (without acoustic factor applied) compared with Pentacam and Galilei when measuring thinner corneas (below approximately 470 μm), whereas these values were overestimated by Orbscan II when measuring the thicker corneas.

The repeatability of pachymetry and keratometry measurements has importance when determining whether there has been progression in keratoconus. Test measurements with poor reproducibility make the detection of
true progression more challenging owing to difficulty in separating the test-retest variability from true change in measurements. Based on the results of this study, the higher test-retest variability of the Orbscan II measurements would require a significantly higher threshold for change to be considered genuine progression compared with Pentacam HR and Galilei.

 Differences in the test-retest variability between instruments may be owing to differences in technology used, such as different optical principles and light sources used by the devices. However, other factors may also account for these differences. In particular, the determination of acceptable scan quality may be a major factor. Whereas Galilei and Pentacam HR provide detailed information regarding the quality of scans, Orbscan II provides little information and automatically discards measurements deemed to be of unacceptable quality. The scan acquisition time also varies between instruments, with a longer acquisition time requiring longer periods without blinking, which may increase corneal irregularity and/or artifacts owing to tear film evaporation.

Contact ultrasound has often been referenced as the standard to which newer modalities have been compared. However, there are significant limitations in using contact ultrasound as a reference, including different operating modes of various ultrasounds, probe design and sensitivity, effects of the contact procedure (topical anesthetic and corneal indentation), nonperpendicular probe position, and variability in the exact location of corneal measurement. Measurements of CCT obtained with the Pentacam have been shown to have higher repeatability and interexaminer correlation compared with ultrasound pachymetry for eyes with keratoconus.

Orbscan slit-scanning pachymetry has been compared to ultrasound in many studies, with variable and limited agreement. Overall, the Orbscan has been found to yield higher readings for corneal thickness (approximately 8% higher) compared with ultrasound. As a result, an acoustic (correction) factor of 0.92 was introduced by the manufacturer to better align Orbscan pachymetry measurements with those obtained by ultrasound. Such a correction factor can be derived by dividing average ultrasound pachymetry by average Orbscan pachymetry measurements of the same corneas. The Orbscan software may incorporate this factor automatically as the default output or allow an alternative factor to be used, and clinicians should be aware of this setting.

Application of the acoustic factor to Orbscan measurements resulted in pachymetry values much lower than both Pentacam and Galilei. It has previously also been shown that for eyes with keratoconus, Orbscan II measurements (AF corrected) underestimate CCT compared to ultrasound. This has clinical implications, as some eyes may not qualify for treatments such as corneal cross-linking based on adjusted pachymetry data from Orbscan, but would qualify without the acoustic factor applied. In this study, Orbscan pachymetry data more closely correlated with Pentacam and Galilei when the acoustic factor was not applied. Because the acoustic factor of 0.92 was derived using normal corneas, this factor may not be accurate when measuring thinner, ectatic corneas.

The Orbscan II uses optical pachymetry and relies on reflections and a clear optical pathway to obtain precise measurements. It has been shown that corneal haze can affect the scanning light system of Orbscan II, resulting in pachymetry measurements that are lower than those obtained using ultrasound. One possible explanation for the differences in pachymetry measurements between Orbscan II and Pentacam HR or Galilei could be the presence of subclinical corneal haze or opacification. To test this hypothesis, we tested for any correlations between subclinical corneal opacification (as measured by Pentacam densitometry) and variability in measurements (pachymetry and pachymetry) within devices. We also tested for any correlations between corneal densitometry and the magnitude of differences in measurements between devices. We did not detect any significant correlations, suggesting that degree of corneal clarity (as measured by Pentacam densitometry scores) was not a significant factor affecting the variability of measurements or differences between devices.

There are several possible limitations of this study. Inter- and interexaminer variability were not investigated and would be expected to also contribute to testing variability. Also, because measurements may be more variable with more severe keratoconus, the findings from this study may not be applicable to other cohorts of keratoconic eyes with more or less severe disease. However, the eyes included in this study had a wide range of severity of keratoconus.

Another limitation is the inclusion of some eyes with suboptimal scan quality. We did not exclude any eyes, even those with lower-quality scans, in order to provide data more representative of measurements obtained in a typical clinical setting. When a higher cutoff of 85% for the quality score was used with the Galilei, analysis (not shown) demonstrated less variability in the measurements, as would be expected. In practice, it is often difficult to obtain high-quality scans of some eyes with severe keratoconus, regardless of the number of attempted scans. Some of the reasons for inability to obtain an acceptable quality score include severe disease causing difficulty obtaining alignment, poor patient fixation, blinking, dry eyes, or other corneal irregularity. It has been demonstrated that performing more measurements/scans within a session increases the sensitivity to detect change between testing sessions. We chose to perform analysis on 3 scans because this is a reasonable number of scans that could be performed in clinical practice. To limit any influence of the normal diurnal variations of corneal curvature and pachymetry, all measurements were performed within a 30-minute time period.
In summary, kerometry and pachymetry measurements cannot be used interchangeably between the studied tomographs in eyes with keratoconus. There are differences in repeatability of measurements taken with all 3 devices, with Orbscan II showing the lowest repeatability. These findings have clinically relevant implications when using different tomographs for the diagnosis, monitoring, and treatment of corneal disease such as keratoconus.

FUNDING/SUPPORT: NO FUNDING OR GRANT SUPPORT. FINANCIAL DISCLOSURES: THE FOLLOWING AUTHORS HAVE NO financial disclosures: Jay J. Meyer, Atelesh Golak, Hans R. Vellaza, Zak Prime, and Charles N.J. McGhee. All authors attest that they meet the current IGME criteria for authorship.

REFERENCES


Appendix C – Screenshot of Web-Based Digital Survey for ARK Part I
Appendix D – Recruitment Material for ARK Parts I and II: A. Total Vision Care
NZAO Newsletter October 2013 and B. CCLS Newsletter January 2014

The Aotearoa Research into Keratoconus

Is Keratoconus more prevalent in New Zealand than other parts of the world?
Does it occur more frequently in particular ethnic groups?
Is the level of severity greater at presentation and does the disease progress at a greater rate than reported internationally?

The Aotearoa Research into Keratoconus or ARK Study will attempt to answer these and other questions concerning keratoconus in New Zealand, through two prospective longitudinal studies run by the Department of Ophthalmology at The University of Auckland.

The first of the two studies (Ark Study Part I) is aimed primarily at determining the epidemiological characteristics of keratoconus in New Zealand, whereas the second (ARK Study: Part II) aims to describe the severity of the disease at presentation and the natural course of the disease. Both studies will be run simultaneously over the next 3 years.

NZAO Members are invited to participate

It is hoped that the NZAO and its members will play a vital role in both parts of the ARK study.

ARK Study: Part I

For the ARK Study: Part I, a secure online survey tool has been developed with the aim of collecting anonymised epidemiologic and clinical data on patients with keratoconus, from all practising Optometrists in New Zealand. This will enable the investigators to obtain important information regarding the incidence and prevalence of the disease in a prospective, longitudinal fashion, from Optometrists, who manage patients with mild to moderate keratoconus. This subset of patients is likely to make up the majority of the population affected by keratoconus. In order to obtain an accurate estimate of the incidence and prevalence, participating Optometrists will be asked to upload information on every patient with keratoconus that undertakes a consultation with them.

It is expected that it will take no more than 2 minutes per patient for a practitioner to upload data, thus we hope this will not be a very time consuming task. A paper alternative to online survey will be available to those who prefer that reporting method.

Both forms of the survey will request the same information; established or new diagnosis of keratoconus, date of birth, ethnicity, gender, type of refractive correction, uncorrected and best corrected visual acuity and keratometry and corneal topography. Practitioners will not be requested to provide any data that could identify their patients.

ARK Study: Part II

The ARK Study: Part II is a natural history study of keratoconus and is similar in design to the Collaborative Longitudinal Evaluation of Keratoconus (CLEK) Study and the Dundee University Scottish Keratoconus Study (DUSKS). This part of the ARK project will be based in Auckland at the University of Auckland clinical research facility located within the Ophthalmology Department at Greenlane Clinical Centre.

The ARK Study: Part II is an observational, prospective longitudinal study, where participants with a new diagnosis of keratoconus will be followed over a period of 16 months over which time, 3 visits - a baseline visit and 2 follow up visits, each separated by 8 months – will be conducted. At each visit advanced ocular imaging technology will be used to quantify the phenotypic features of the cornea and allow for an assessment of how these features are altered as the disease progresses.

The imaging technologies will include: in vivo confocal microscopy, anterior segment optical coherence tomography, analysis of corneal biomechanical properties and computerised corneal tomography. These imaging technologies have not previously been used in this combination in a prospective natural history study of keratoconus and we believe this will provide the most comprehensive insight into the natural course of the disease to date.

Patients in the Auckland Region with a new diagnosis of keratoconus and no previous contact lens wear for more than 3 months will be recruited to participate in the study. Considering that patients with keratoconus are usually diagnosed first by their optometrist, the best avenue for recruiting patients that meet the study’s criteria is through optometrists in the Auckland Region, who will be offered the opportunity to refer patients to participate in the study.

It is important to note that we are conducting observational research: we wish to assess a patient before
(ARK) Study

Contact lenses are fitted but once the first research visit occurs the clinical management of the patient is the sole responsibility of the referring practitioner although we can provide details of all our investigations.

The ARK Study is a cross-disciplinary project being undertaken in the Department of Ophthalmology, University of Auckland, involving both optometrists and ophthalmologists. The study includes a PhD project in ophthalmology being undertaken by Akhilesh Gokul, a New Zealand trained, registered and practicing optometrist (TPA), being supervised by Professor Charles McGhee and Associate Professor Dipika Patel. There will also be significant involvement from Stuti Misra, a Post-Doctoral Clinical Research Fellow. In addition, it is also likely that in 2014 another New Zealand trained and registered optometrist will be joining the ARK team, and he/she will conduct doctoral level research on other facets of the enigma that is keratoconus.

Anticipated outcomes

The ARK Study Part I aims to document how many people in New Zealand are living with keratoconus and how many new cases are diagnosed each year. This will allow governments and district health boards to make better informed decisions when allocating resources now and in the future.

The ARK Study Part II aims to provide a comprehensive insight into the natural history of keratoconus, thereby enhancing our knowledge of the disease and potentially identifying risk factors for progression. This may have a profound impact on clinical practice, allowing clinicians to employ evidence-based practice to identify patients with a greater risk of progression and monitoring more closely or treating these patients as required. In addition, information on the level of disease severity and progression, will further inform resource allocation decisions made by district health boards around New Zealand.

CPD component

Participating Optometrists who provide epidemiologic data in Part I, refer patients to enrol in Part II, or both will not only make an invaluable contribution to advancing the current understanding of keratoconus, they will also receive access to an exclusive online CPD point programme, available only to participating practitioners.

We encourage all NZAO members to get involved in this exciting new project, please email: thearkstudy@mail.com, with your name and place of practice, if you would like to participate or with any other enquires if you would like more information on either part of the project.

References:


The Aotearoa Research into Keratoconus (ARK) Study – Launched!

Is Keratoconus more prevalent in New Zealand than other parts of the world? Does it have a predilection for particular ethnic groups? Is its severity greater at presentation and does the disease progress at a greater rate than reported internationally? The Aotearoa Research into Keratoconus or ARK Study will attempt to answer these and other questions concerning keratoconus in New Zealand, through two prospective longitudinal studies run by the Department of Ophthalmology, University of Auckland. Both the ARK Study: Part I and the ARK Study: Part II have now been launched and are in the data collection phase!

It is hoped that alongside the NZAO, the Cornea and Contact Lens Society of New Zealand and its members will play a vital role in both parts of the ARK study. The ARK Study: Part I aims to elucidate the incidence, prevalence, demographic and clinical characteristics of patients with keratoconus in New Zealand. The study is focused on patients managed by Optometrists and consists of a survey that will hopefully be filled in by every Optometrist in the country following every consultation with a patient with keratoconus in the next 2-3 years. The survey should only take 2-4 minutes to complete for each patient, hence it is not expected to be a very time consuming task. A paper alternative is also available. Both forms of the survey request the same information; established or new diagnosis of keratoconus, date of birth, ethnicity, gender, type of refractive corrections, uncorrected and best corrected visual acuity and keratometry or corneal topography.

There has already been a fantastic response through the NZAO drive, with nearly 80 Optometrists nationwide having expressed interest in participating in the ARK Study: Part I, however, it is not too late to enrol. Please email, thearkstudy@gmail.com, with your name and place of practice, to enrol. We will reply with a participant information sheet (PIS) detailing the study, and consent form that you will need to sign. Once you return the signed consent form, a password and link to the online survey or the paper form of the survey will be provided. You can then begin data collection.

The ARK Study: Part II is an observational, prospective longitudinal study, where participants with a new diagnosis of keratoconus will be followed over a period of 16 months over which time, 3 visits - a baseline visit and 2 follow up visits, each separated by 8 months - will be conducted. At each visit advanced ocular imaging technology will be used to quantify the phenotypic features of the cornea and allow for an assessment of how these features are altered as the disease progresses. These imaging
technologies will include; in vivo confocal microscopy, anterior segment OCT, analysis of corneal biomechanical properties and computerised corneal tomography. Participants will consist of patients in the Auckland Region with a new diagnosis of keratoconus and no previous contact lens wear for more than 3 months. The ARK Study: Part II will be based in Auckland at the University of Auckland clinical research facility located within the Ophthalmology Department at Greenlane Clinical Centre. It is important to note that we are only conducting observational research and that clinical management of the patient is the responsibility of the referring practitioner, although we can provide details of all our investigations.

We would very much appreciate the assistance of Optometrists in the Auckland Region in recruiting participants. Please inform all patients with a new diagnosis of keratoconus and previous contact lens wear of no more than 3 months, of the nature of the study and the petrol voucher incentive. If the patient consents, please send their contact details to us via email (thearkstudy@gmail.com), post or fax. We will then contact them directly to assess if they meet the study criteria and to arrange an appointment. It is not necessary to enrol for the ARK Study: Part II, but please email us if you would like more information on the study.

ARK is a cross disciplinary project involving both Optometrists and Ophthalmologists. The project will form part of PhD being undertaken by Akilesh Gokul, a New Zealand trained, registered and practicing Optometrist, being supervised by Professor Charles McGhee and Associate Professor Dipika Patel, with significant involvement from Dr Stuti Misra, a Post-Doctoral Clinical Research Fellow.

Participating Optometrists who provide data in Part I, refer patients to Part II, or both will make an invaluable contribution to advancing the current understanding of keratoconus, and will also receive access to an exclusive online CPD point programme, available only to participating practitioners in Autumn/Winter 2014.
### More Results from Equipment Survey

<table>
<thead>
<tr>
<th>Equipment Survey</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIO</td>
<td>169</td>
</tr>
<tr>
<td>Accommodation rule</td>
<td>112</td>
</tr>
<tr>
<td>Colour vision test</td>
<td>191</td>
</tr>
<tr>
<td>Distance and near oculomotor balance tests</td>
<td>169</td>
</tr>
<tr>
<td>Test for stereopsis</td>
<td>190</td>
</tr>
<tr>
<td>Applanation tonometer (non-contact and contact)</td>
<td>188</td>
</tr>
<tr>
<td>Gonioscope</td>
<td>181</td>
</tr>
<tr>
<td>Amsler's charts</td>
<td>192</td>
</tr>
<tr>
<td>Slit lamp biomicroscope</td>
<td>191</td>
</tr>
<tr>
<td>Slit lamp photography</td>
<td>99</td>
</tr>
<tr>
<td>Condensing lens for indirect ophthalmoscopy with the slit lamp</td>
<td>183</td>
</tr>
<tr>
<td>Keratometer</td>
<td>175</td>
</tr>
<tr>
<td>Pen torch</td>
<td>182</td>
</tr>
<tr>
<td>Contrast sensitivity test chart</td>
<td>106</td>
</tr>
<tr>
<td>Foci meter</td>
<td>148</td>
</tr>
<tr>
<td>Supplementary vision charts (e.g. Low vision charts, Sheridan Gardiner, Landolt C etc)</td>
<td>136</td>
</tr>
<tr>
<td>Refractor head</td>
<td>163</td>
</tr>
<tr>
<td>Auto refractor</td>
<td>98</td>
</tr>
<tr>
<td>Lensometers</td>
<td>143</td>
</tr>
<tr>
<td>Sphygmomanometer</td>
<td>27</td>
</tr>
<tr>
<td>Contact lens fitting set</td>
<td></td>
</tr>
<tr>
<td>Soft, soft toric, soft multifocal</td>
<td>163</td>
</tr>
<tr>
<td>Rigid gas permeable</td>
<td>138</td>
</tr>
<tr>
<td>Specialty, i.e. Post graft and Keratoconus</td>
<td>105</td>
</tr>
</tbody>
</table>
Other Cited Material


B. Data concerning registered, practicing optometrists in New Zealand obtained through personal correspondence (AG) with the Optometrists and Dispensing Opticians Board New Zealand

C. Equipment survey result available in the NZAO Total Vision Care newsletter for September 2015 (Appendix D)

References


