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 recognise 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.

To request permissions please use the Feedback form on our webpage.
http://researchspace.auckland.ac.nz/feedback

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.
Keratoconus: novel investigations in the analysis of the cornea in the diseased state

Jennifer Chen-Chia Fan

BHB, MBChB

A thesis submitted in partial fulfillment of the requirements of the degree of

Doctor of Medicine

Department of Ophthalmology

University of Auckland

2014
Abstract

Keratoconus is a relatively common, potentially blinding, disease of the cornea. Although clinically defined more than 150 years ago, the disease is still not well-understood. In New Zealand, keratoconus has a higher prevalence than elsewhere in the world, frequently presents to ophthalmology services at an advanced stage, and is the most common indication for corneal transplantation.

The inter-related series of studies comprising this thesis was therefore developed to investigate two poorly understood areas associated with keratoconus: the natural history of acute corneal hydrops and the measurement/variation of intraocular pressure (IOP) in the disease process.

The current understanding, methods of investigation and management of acute hydrops in keratoconus were defined through a succinct review of the published scientific literature, supplemented by the personal experience and perspective of the author and senior collaborators. This enabled the investigator to create appropriate studies in the context of our knowledge of the disease process and this critical review was published as a “Perspective” in the American Journal of Ophthalmology at the completion of the studies contained in this thesis.

Subsequently, patient demographic details, ocular and medical history, family history, presentation and course of disease were analysed in a large series of keratoconus patients in order to identify the predictors and risk factors for acute corneal hydrops in a New Zealand population. Notably, subjects with hydrops
were more likely to be of Pacific ethnicity and less likely to be of New Zealand European ethnicity. Affected individuals were more likely to have a history of eye-rubbing, but less likely to have a family history of keratoconus. Somewhat paradoxically, such subjects were not identified to have increased likelihood of undergoing corneal transplantation compared to the non-hydrops, keratoconic subjects in the study population.

The *in vivo* confocal microscopy and *ex vivo* microstructural changes associated with acute hydrops were assessed in a comprehensive, longitudinal manner hitherto unreported in the literature. The microstructural changes occurring throughout the course of acute hydrops in keratoconus were documented and analysed using *in vivo* confocal microscopy in a prospective study of ten subjects. This study identified unusual cells in the epithelium and stroma of four corneas and in two corneas, which subsequently developed neovascularisation, large stromal cells with branching processes persisted until three months post-presentation.

The corneal buttons of five of these ten subjects with acute hydrops were subsequently collected, following penetrating keratoplasty, for *ex-vivo* immunohistochemical and histological analyses. This latter study highlighted pronounced inflammatory and fibrotic changes following acute corneal hydrops, and a novel finding of dendritic cells in the endothelial layer.

The majority of subjects with advanced keratoconus and acute hydrops progress to corneal transplantation. Anecdotally subjects with keratoconus have been
reported to have a greater risk of corticosteroid-related elevation of IOP. The incidence of post-keratoplasty IOP elevation in keratoconus and the relationship to corticosteroid administration was therefore assessed in 48 subjects. The incidence of IOP elevation post-surgery in this keratoconic population was higher than reported elsewhere (35%) and was presumed to be steroid-related. Interestingly, IOP elevation was less likely to occur in Maori/Pacific peoples compared to New Zealand Europeans post-keratoplasty.

In relation to IOP measurement in the setting of abnormal corneal thinning and shape as occurs in keratoconus, the new Corvis Scheimpflug Tonometer was compared to Goldmann applanation tonometry, rebound tonometry and dynamic contour tonometry in the assessment of IOP measurement in keratoconic eyes. Among the instruments, the Corvis tonometer and the Goldmann tonometer demonstrated the least agreement in keratoconic eyes. IOP obtained by the Corvis tonometer was strongly associated with deformation amplitude of the cornea. Evidence suggests that none of the devices should be used interchangeably in measuring IOP in abnormal corneas such as those exhibiting keratoconus.

In conclusion, the series of studies that constitute this thesis have significantly increased our insight into what is still a relatively enigmatic disease: keratoconus. These studies have particularly considered the disease as it manifests in New Zealand and in the New Zealand populations. The data presented highlight relatively late and severe presentation with acute hydrops in Pacific and Maori populations. In addition to analyses of aetiological associations, a longitudinal in
*vivo* confocal microscopy clinical study has provided a unique perspective on the microstructural features of hydrops, and this has been further consolidated by extensive histological analyses of corneal buttons excised at corneal transplantation. Inflammatory changes were identified during the course of hydrops that may predispose to neovascularisation, and some inflammatory processes persist even in the clinically “quiescent stage” prior to corneal transplantation. Following corneal transplantation it appears that patients with keratoconus are more likely to develop corticosteroid-related elevation of IOP than previously reported. An analysis of methods of IOP assessment in keratoconus highlights the benefits and limitations of contemporary devices and that these cannot be used interchangeably in this disease.

While a number of future research questions have been raised in the process of these inter-related studies, I hope that the foundation upon which subsequent research is built has been significantly augmented by this work on keratoconus, corneal hydrops and the assessment of IOP in this disease.
Acknowledgements

Completion of this thesis would not have been achieved without the contribution, guidance and support so generously offered to me by several individuals.

First and foremost I would like to thank Professor Charles McGhee for the opportunity to undertake this research degree under his supervision at the Department of Ophthalmology, University of Auckland. He has supported my career in ophthalmology since my time as a medical student. Were it not for his tremendous input, encouragement and mentorship, this thesis would not have come to fruition. He is involved in every study comprised in this thesis, and his support provided the backbone to this journey.

I would also like to thank Associate Professor Trevor Sherwin for his co-supervision on the laboratory sections of my thesis. He was always available for me to discuss ideas, seek reassurance, or simply to vent a frustration. His saintly patience was demonstrated no better than when faced with my “unconventional” lab-book keeping skills. I am extremely grateful for his continued support and friendship.

Associate Professor Dipika Patel has been an invaluable resource during the completion of this thesis. She introduced me to the various anterior segment imaging technologies utilised in this thesis and in particular taught me how to use the in vivo confocal microscopes. I am incredibly appreciative of her editorial skills and contribution to several of the studies in this thesis.
I would like to express my appreciation to Judy Loh in the Department of Ophthalmology, University of Auckland. She introduced me to the art of corneal sectioning and staining in the laboratory, and never tired of my endless questions. She has contributed significantly to the laboratory component of two of the studies in this thesis.

A special thanks goes to University of Auckland Summer student Dr. William Good, whose dedication during his summer selective in retrieving the data of each individual hydrops patient is immensely appreciated. I would also like to thank Dr. Charlotte Jordan for her assistance in data collection in the “Predictors of Hydrops” study, and Dr. Kent Chow and Dr. Noor Ali, for their invaluable contributions to the section on ‘Studies of intraocular pressure in keratoconus’.

Clinical research by its nature typically requires an integrated team approach and I wish to thank all those in the Departments of Ophthalmology in the University of Auckland and Auckland District Heath Board who contributed to the clinical care of the patients included in the various studies contained in this thesis.

I would like to acknowledge the Health Research Council (HRC) of New Zealand for providing me with the generous Clinical Research Training Fellowship - Career Development Award. This Award not only enabled me to take eighteen months out of full-time clinical duties in order to conduct the research required for this thesis, their recognition of the value of my work also provided immeasurable encouragement.
Finally, I would like to thank my parents, Samuel and Miriam Fan, who taught me that anything is possible, and my husband, Brent Gaskin, who makes everything possible.

Jennifer C.C. Fan

Auckland, 8th January 2014
# Table of Contents

**Section I: Introduction**

- Chapter 1: Anatomy of the human cornea 2
- Chapter 2: Physiology and function of the cornea 12
- Chapter 3: Measuring intraocular pressure using contemporary corneal-based techniques 22

**Section II: Studies on keratoconus and acute corneal hydrops** 58

- Chapter 4: Acute hydrops in keratoconus: new perspectives 59
- Chapter 5: Predictors of acute corneal hydrops in keratoconus: the Auckland keratoconus study 83
- Chapter 6: A prospective study of microstructural changes in acute corneal hydrops in keratoconus using *in vivo* confocal microscopy 103
- Chapter 7: Immunohistochemical analyses of excised corneal tissue following acute hydrops in keratoconus 126
- Chapter 8: Keratoconus and keratoconus surgery-related case studies
  - a. Long-term corneal microstructural changes following epikeratophakia for aphakia: *in vivo* confocal microscopic analysis of an uncommon technique 151
previously employed in keratoconus

b. Clinical and histological manifestations of an extreme Descemet’s membrane tear in acute corneal hydrops

Section III: Studies of intraocular pressure in keratoconus

Chapter 9: Intraocular pressure elevation in subjects with keratoconus that have undergone corneal transplantation

Chapter 10: Measurement of IOP in keratoconus: comparison of Corvis Scheimpflug tonometer with Goldman applanation, rebound, and dynamic contour tonometry

Section IV: Conclusions

Chapter 12: Conclusions: Novel investigations of keratoconus and its assessment in New Zealand

Section V: Appendices

Appendix 1: Peer-reviewed papers originating from this thesis

Appendix 2: Thesis related conference presentations and posters

x
## List of tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>Subject characteristics</td>
<td>91</td>
</tr>
<tr>
<td>7.1</td>
<td>Panel of immunohistochemical antibodies</td>
<td>130</td>
</tr>
<tr>
<td>7.2</td>
<td>Demographic and clinical data</td>
<td>133</td>
</tr>
<tr>
<td>8.1.1</td>
<td>Mean cell densities and sub-basal nerve densities of the epikeratophakic cornea and the normal contralateral cornea</td>
<td>157</td>
</tr>
<tr>
<td>9.1</td>
<td>Incidence of Post-keratoplasty pressure elevation over 12-month follow-up in keratoconus</td>
<td>199</td>
</tr>
<tr>
<td>9.2</td>
<td>Treatment methods used to managed elevated IOP from 1-week to 12-months post-keratoplasty</td>
<td>202</td>
</tr>
<tr>
<td>9.3</td>
<td>Percentage of post-keratoplasty elevated IOP in keratoconus in comparison with other studies</td>
<td>204</td>
</tr>
<tr>
<td>10.1</td>
<td>Mean intra-ocular pressure measurements by the Corvis ST compared to Goldmann applanation tonometer, ICare rebound tonometer and Pascal dynamic contour tonometer in normal and keratoconic eyes</td>
<td>220</td>
</tr>
<tr>
<td>10.2</td>
<td>Pearson's correlation agreement of intraocular pressure measurements in 4 tonometers with various corneal parameters in in normal and keratoconic eyes</td>
<td>225</td>
</tr>
<tr>
<td>10.3</td>
<td>Multiple regression analysis assessing the correlation between various corneal parameters and IOP measured by Goldmann applanation tonometer and Corvis ST in keratoconic eyes.</td>
<td>226</td>
</tr>
</tbody>
</table>
## List of figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Cross-section of the normal human cornea</td>
<td>4</td>
</tr>
<tr>
<td>1.2</td>
<td>A montage of in vivo confocal microscopy images of the normal corneal sub-basal plexus</td>
<td>10</td>
</tr>
<tr>
<td>2.1</td>
<td>Mechanism of electrolyte and water secretion of the lacrimal gland acinar cell</td>
<td>15</td>
</tr>
<tr>
<td>2.2</td>
<td>Model of ion and water transport across the corneal endothelium</td>
<td>20</td>
</tr>
<tr>
<td>3.1</td>
<td>Goldmann applanation tonometer</td>
<td>25</td>
</tr>
<tr>
<td>3.2</td>
<td>The TonoPen</td>
<td>28</td>
</tr>
<tr>
<td>3.3</td>
<td>The iCare rebound tonometer</td>
<td>30</td>
</tr>
<tr>
<td>3.4</td>
<td>Profiles corresponding to an ORA measurement of IOP and corneal biomechanical properties</td>
<td>36</td>
</tr>
<tr>
<td>4.1</td>
<td>Severe acute hydrops in keratoconus</td>
<td>63</td>
</tr>
<tr>
<td>4.2</td>
<td>Resolved corneal hydrops in keratoconus</td>
<td>64</td>
</tr>
<tr>
<td>4.3</td>
<td>Anterior segment optical coherence tomography of resolved corneal hydrops in keratoconus</td>
<td>68</td>
</tr>
<tr>
<td>4.4</td>
<td><em>In vivo</em> confocal microscopy of cellular changes in acute hydrops in keratoconus</td>
<td>71</td>
</tr>
<tr>
<td>5.1</td>
<td>Severe acute hydrops in keratoconus</td>
<td>92</td>
</tr>
<tr>
<td>5.2</td>
<td>Severe corneal hydrops in keratoconus with corresponding in vivo confocal microscopy images</td>
<td>94</td>
</tr>
<tr>
<td>5.3</td>
<td>Resolved corneal hydrops in keratoconus</td>
<td>96</td>
</tr>
<tr>
<td>Section</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>------------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>5.4</td>
<td>Histology of persistent Descemet’s detachment following corneal hydrops</td>
<td>98</td>
</tr>
<tr>
<td>6.1</td>
<td>Clinical images of acute corneal hydrops in 10 eyes at initial presentation and at resolution</td>
<td>110</td>
</tr>
<tr>
<td>6.2</td>
<td><em>In vivo</em> confocal microscopy images of corneal epithelial bullae in the superficial and wing cell layers during acute corneal hydrops</td>
<td>114</td>
</tr>
<tr>
<td>6.3</td>
<td><em>In vivo</em> confocal microscopy images of keratocytes and intercellular lacunae</td>
<td>115</td>
</tr>
<tr>
<td>6.4</td>
<td><em>In vivo</em> confocal microscopy images showing cellular and extracellular changes occurring during acute corneal hydrops</td>
<td>117</td>
</tr>
<tr>
<td>6.5</td>
<td><em>In vivo</em> confocal microscopy images of microfolds in the anterior and mid stroma</td>
<td>118</td>
</tr>
<tr>
<td>6.6</td>
<td><em>In vivo</em> confocal microscopy images of unusual cellular structures demonstrated in corneas during acute hydrops</td>
<td>119</td>
</tr>
<tr>
<td>7.1</td>
<td>H&amp;E-stained sections of post-hydrops corneas</td>
<td>136</td>
</tr>
<tr>
<td>7.2</td>
<td>Immunofluorescent staining of various cellular populations in post-hydrops corneas</td>
<td>139</td>
</tr>
<tr>
<td>7.3</td>
<td>Immunofluorescent staining of cellular and extracellular features in post-hydrops corneas</td>
<td>141</td>
</tr>
<tr>
<td>7.4</td>
<td>High magnification 3D confocal microscopy of laminin deposition in a corneal section post-hydrops</td>
<td>142</td>
</tr>
<tr>
<td>8.1.1</td>
<td>Clinical anterior segment photographs demonstrating corneal details following epikeratophakia</td>
<td>155</td>
</tr>
<tr>
<td>8.1.2</td>
<td><em>In vivo</em> confocal microscopy comparing the grafted</td>
<td>156</td>
</tr>
</tbody>
</table>
lenticule with the normal counterparts in the contralateral
eye (from basal epithelium to the graft-host interface)

8.1.3 *In vivo* confocal microscopy comparing the grafted lenticule
with the normal counterparts in the contralateral
(from the anterior stroma to endothelium)

8.2.1 Clinical, dark field microscopic, and environmental scanning
electron microscopic images of an extreme Descemet's tear

8.2.2 Orbscan tomography of the post-hydrops eye

8.2.3 Orbscan tomography of the contralateral, non-hydrops eye

8.2.4 H&E stained section of excised corneal button

8.2.5 Details images of the Descemet's tear

10.1 Corvis Scheimpflug imaging of the cornea at maximal
concavity during applanation

10.2.1 Bland-Altman plots of the agreement between different
tonometers in the measurement of IOP in eyes with
keratoconus (a-c)

10.2.2 Bland-Altman plots of the agreement between different
tonometers in the measurement of IOP in eyes with
keratoconus (d-e)

10.3.1 Bland-Altman plots of the agreement between different
tonometers in normal eyes (a-b)

10.3.2 Bland-Altman plots of the agreement between different
tonometers in normal eyes (c-d)
10.4 Bland-Altman plots of the agreement in central corneal thickness measurements between the Corvis ST and ultrasound pachymetry in keratoconic and normal eyes
# List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3D</td>
<td>3-dimensional</td>
</tr>
<tr>
<td>Anti-VEGF</td>
<td>Anti- Vascular endothelial growth factor</td>
</tr>
<tr>
<td>AS-OCT</td>
<td>Anterior segment optical coherence tomography</td>
</tr>
<tr>
<td>BCVA</td>
<td>Best-corrected visual acuity</td>
</tr>
<tr>
<td>BM</td>
<td>Basement membrane</td>
</tr>
<tr>
<td>BSCVA</td>
<td>Best spectacle-corrected visual acuity</td>
</tr>
<tr>
<td>CA</td>
<td>Carbonic anhydrase</td>
</tr>
<tr>
<td>CCT</td>
<td>Central corneal thickness</td>
</tr>
<tr>
<td>CH</td>
<td>Corneal hysteresis</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CLEK</td>
<td>Collective longitudinal evaluation of keratoconus</td>
</tr>
<tr>
<td>CRF</td>
<td>Corneal resistance factor</td>
</tr>
<tr>
<td>CST</td>
<td>Corvis Scheimpflug tonometer</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>D</td>
<td>Diop tre</td>
</tr>
<tr>
<td>DA</td>
<td>Deformation amplitude</td>
</tr>
<tr>
<td>DALK</td>
<td>Deep anterior lamellar keratoplasty</td>
</tr>
<tr>
<td>DAPI</td>
<td>4’,6-diamidino-2-phenylindole</td>
</tr>
<tr>
<td>DM</td>
<td>Descemet’s membrane</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>EGF</td>
<td>Epidermal growth factor</td>
</tr>
<tr>
<td>ESEM</td>
<td>Environmental scanning electron microscope</td>
</tr>
<tr>
<td>GAG</td>
<td>Glycosaminoglycan</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>GAT</td>
<td>Goldmann applanation tonometer</td>
</tr>
<tr>
<td>HRT II</td>
<td>Heidelberg Retina Tomograph II</td>
</tr>
<tr>
<td>H &amp; E</td>
<td>Haematoxylin and Eosin</td>
</tr>
<tr>
<td>ICC</td>
<td>Intra-class correlation</td>
</tr>
<tr>
<td>ICT</td>
<td>iCare rebound tonometer</td>
</tr>
<tr>
<td>IHC</td>
<td>Immunohistochemistry</td>
</tr>
<tr>
<td>IOP</td>
<td>Intraocular pressure</td>
</tr>
<tr>
<td>IOPg</td>
<td>Goldmann-correlated IOP</td>
</tr>
<tr>
<td>IOPcc</td>
<td>Corneal-compensated IOP</td>
</tr>
<tr>
<td>IVCM</td>
<td>In vivo confocal microscopy</td>
</tr>
<tr>
<td>KC</td>
<td>Keratoconus</td>
</tr>
<tr>
<td>Kmax</td>
<td>Maximum keratometry</td>
</tr>
<tr>
<td>L</td>
<td>left</td>
</tr>
<tr>
<td>Max-K</td>
<td>Maximum keratometry</td>
</tr>
<tr>
<td>Min-K</td>
<td>Minimum keratometry</td>
</tr>
<tr>
<td>NV</td>
<td>Neovascularisation</td>
</tr>
<tr>
<td>OD</td>
<td>oculus dexter (right eye)</td>
</tr>
<tr>
<td>OPA</td>
<td>Ocular pulse amplitude</td>
</tr>
<tr>
<td>ORA</td>
<td>Ocular response analyser</td>
</tr>
<tr>
<td>OS</td>
<td>oculus sinister (left eye)</td>
</tr>
<tr>
<td>P1</td>
<td>First applanation pressure</td>
</tr>
<tr>
<td>P2</td>
<td>Second applanation pressure</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PDCT</td>
<td>Pascal dynamic contour tonometer</td>
</tr>
<tr>
<td>PKA</td>
<td>Protein kinase A</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>PKP</td>
<td>Penetrating keratoplasty</td>
</tr>
<tr>
<td>PMCD</td>
<td>Pellucid marginal degeneration</td>
</tr>
<tr>
<td>PS</td>
<td>Posterior stroma</td>
</tr>
<tr>
<td>QDS</td>
<td>quater die sumendum (four times daily)</td>
</tr>
<tr>
<td>R</td>
<td>right</td>
</tr>
<tr>
<td>RCM</td>
<td>Rostock corneal module</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>UBM</td>
<td>Ultrasound biomicroscopy</td>
</tr>
<tr>
<td>UCVA</td>
<td>Uncorrected visual acuity</td>
</tr>
</tbody>
</table>
Section 1

*Introduction*
Chapter 1

Anatomy of the Cornea
1.1 Introduction

The cornea is a transparent, avascular tissue that forms the anterior one-sixth of the outer fibrous coat of the eyeball. It has both optical and protective properties. The cornea acts like a window allowing light to transmit to the retina, and it forms the principal refractive surface of the eye. The cornea also provides a mechanically resilient, and chemically resistant barrier between the eye and the environment.

1.2 Gross anatomy

The average corneal diameter is 11.7 mm horizontally and 10.6 mm vertically,\(^1\) giving an elliptical appearance. When viewed posteriorly, the cornea is circular with an average diameter of 11.7 mm.\(^1\) It is 540 µm thick centrally, and becomes thicker towards the periphery as it flattens, measuring 670 µm.\(^2\) The central third of the anterior surface (the optical zone, 4 mm diameter) is approximately spherical with a radius of curvature of 7.8 mm anteriorly and 6.5 mm posteriorly.\(^2\) This area provides the majority of optical function of the cornea.\(^3\)

The periphery of the cornea is continuous with the sclera (remaining five-sixth of the fibrous outer coat of the eyeball), episcleral tissue and conjunctiva, and they merge in a 1-2 mm transitional zone named the limbus.\(^2\) The limbus is rich in vascular supply as it requires substantial support to maintain epithelial turnover (see next section).\(^4\) The anterior surface of the cornea is coated by the tear film and the posterior surface is bathed in aqueous humor.
1.3 Microscopic anatomy

The cornea is composed of five distinct tissue layers: 1) epithelium, 2) Bowman’s layer, 3) stroma, 4) Descemet’s membrane, and 5) endothelium (fig 1.1).

Figure 1.1 Histological cross-section of the cornea stained with haematoxylin and eosin (H&E) (Courtesy of Professor Charles McGhee)

Epithelium

The corneal epithelium is composed of stratified, squamous, and nonkeratinised epithelial cells and accounts for ten percent of the total corneal thickness. It measures approximately 50-60 µm thick. The epithelium consists of 3-4 layers of
superficial flattened squamous cell layers, 1-3 mid-epithelial ‘wing’ cell layers, and 1 basal cell layer.\textsuperscript{6,7}

Superficial cells are connected by desmosomes, forming a semipermeable membrane, preventing tear fluid from penetrating the stroma.\textsuperscript{2} The basal epithelial cells form the germinative layer of the corneal epithelium, where mitoses of the basal epithelial cells allow for complete turnover of the entire epithelial layer every 5-7 days.\textsuperscript{8} The daughter cells migrate anteriorly, differentiating into wing and squamous cells before desquamation from the apical surface. Basal cells are renewed by the centripetal migration of new basal cells originating from limbal stem cells.\textsuperscript{8} Hemidesmosomal structures firmly attach the columnar basal cells to the basal lamina.\textsuperscript{7}

The basal lamina is secreted by the basal epithelial cells during embryonic development and throughout adult life.\textsuperscript{2,9} It is a thin network of collagen (type IV and VII) and laminin, and more strongly adherent to Bowman’s layer and the stroma posteriorly than to the epithelium.\textsuperscript{10,11} It forms a boundary between the epithelium and stroma and provides a scaffold for the organization of the epithelium.\textsuperscript{9}

\textbf{Bowman’s layer}

Bowman’s layer is an acellular homogenous zone, 8-14 µm thick lying immediately subjacent to the basal lamina of the corneal epithelium.\textsuperscript{12} It consists of randomly-oriented, tightly- packed collagen fibrils.\textsuperscript{13} The collagen fibrils of Bowman’s layer
are half to two-thirds as thick as the fibrils of the underlying stroma, and are primarily composed of collagen types I, III and V and associated proteoglycans densely woven into a felt-like matrix.\textsuperscript{14} In the posterior region of this layer the collagen fibrils become progressively more orderly, blending with those of the anterior stroma.\textsuperscript{12,14,15} Bowman’s layer is perforated in many places by unmyelinated nerves in transit to the corneal epithelium.\textsuperscript{14}

Bowman’s layer has several functions: it provides a strong barrier against infective agents and surface tumours; it provides resistance to mechanical trauma; and plays a role in maintaining the smooth anterior surface of the cornea.\textsuperscript{16}

**Stroma**

The stroma of the cornea is a dense connective tissue layer that constitutes approximately 90\% of the corneal thickness and is normally 78\% dehydrated.\textsuperscript{17} The stroma is composed primarily of 200-300 collagen lamellae, proteoglycan ground substance, and collagen-producing fibroblasts, known as keratocytes.\textsuperscript{18} The predominant collagen in the stroma is type I (50-55\%), with smaller amounts of type III, V, and VI.\textsuperscript{13} The diameter of each collagen fibril is small (30 nm) and consistent, with uniform interfibrillar distance.\textsuperscript{13,19} The collagen fibrils make up lamellae that run parallel to the corneal surface anteriorly and at right angles to those in consecutive layers posteriorly.\textsuperscript{20} These highly characteristic features are essential for the cornea to maintain transparency and tensile strength. This will be discussed further in Chapter 2.
The ground substance in which the collagen fibrils are embedded has an important role in maintaining collagen interfibrillar distance.\textsuperscript{13} The major corneal proteoglycans are decorin (associated with dermatan sulfate) and lumican (associated with keratin sulfate).\textsuperscript{13}

There are 2.0-3.5 million keratocytes in the human cornea and they occupy as much as 9 to 17\% of total stromal volume.\textsuperscript{21} They are responsible for the production of collagen, proteoglycans and glycoproteins.\textsuperscript{13} They are located predominantly between collagen lamellae and are long, flattened, stellate cells arranged into a syncytium, forming a communication network through their branching processes.\textsuperscript{22}

**Descemet's membrane**

Descemet's membrane is the basement membrane of the corneal endothelium. It is primarily composed of type IV collagen, with lesser amounts of type V, VIII, IX and XII surrounded by supportive glycoproteins.\textsuperscript{23}

It is secreted by the endothelium and first appears in the second month of gestation. Its thickness increases throughout life from 3-4 µm at birth to 10-12 µm by late adulthood.\textsuperscript{12} Two distinct zones can be seen on electron microscopy: the banded anterior one-third is produced in fetal life and the posterior two-thirds is formed after birth and has a homogenous granular structure.\textsuperscript{24} It is the posterior zone that thickens with age without apparent destruction of previously formed
layers. Descemet’s membrane is not strongly attached to the stroma and can be detached in pathological conditions or dissected surgically.

Endothelium

The human corneal endothelium is not a true endothelium; it is derived from the neural crest and therefore of neuroectodermal rather than vascular origin. It is a single layer of flattened cells, which line the posterior corneal surface. These cells are hexagonal en face and cuboidal in cross-section. They measure 4-6 µm thick and 20 µm in diameter. The cells are firmly bound together by occluding junctions and communicate extensively through numerous gap junctions. The basal cell membrane is attached to Descemet’s membrane by modified hemidesmosomes. The apical cell membrane is relatively flat and exhibits microvilli, the function of which is unknown.

The endothelial cells are occupied internally by a large central nucleus and numerous organelles, including mitochondria, endoplasmic reticulum and Golgi’s apparatus. These reflect the high metabolic activity of endothelial cells.

At birth, the human cornea has a fixed population of approximately 6000 cells/mm². Although mitosis can occur in young endothelial cells, the number of cells declines rapidly in the first five years to reach a value of 3500 cells/mm². In adulthood, endothelial cell loss occurs at a rate of approximately 0.6% per year and is not replaced. Nor are the cells replaced after injury and instead adjacent cells enlarge and spread to cover the area of loss (see Chapter 2). An increase
in size variation (polymegathism) and shape variation (polymorphism) also occur with age.\textsuperscript{30}

\section*{1.4 Corneal Innervation}

The cornea is one of the most densely innervated tissues in the body, 20-40 times more sensitive than tooth pulp and 300 times more sensitive than skin.\textsuperscript{32} The sensory innervation of the cornea is supplied by the ophthalmic division of the trigeminal nerve via the long ciliary nerves.\textsuperscript{33-35} Sympathetic innervation has been described in rabbit and cat corneas from the superior cervical ganglion, however, sympathetic fibres are found to be exceedingly scarce in human corneas.\textsuperscript{35}

Approximately 60-80 myelinated branches, and fewer finer, unmyelinated branches, of the long ciliary nerves enter the anterior corneal stroma at the corneoscleral limbus.\textsuperscript{36} After 1.0 – 1.5 mm the myelinated fibres lose their myelin sheaths and divide into two groups – anterior and posterior.\textsuperscript{36} They continue to branch dichotomously towards the corneal centre.\textsuperscript{37} A close relationship has been demonstrated between keratocytes and the nerve fibres.\textsuperscript{38,39}

The anterior nerves (40-50) pass through the substance of the stroma and form a dense subepithelial plexus. From this plexus, the nerves turn 90\textdegree{} and penetrate Bowman’s layer, shedding their Schwann cell sheaths as they do so. They then turn 90\textdegree{} again after penetrating Bowman’s layer, running parallel to the corneal surface they divide several times to become the sub-basal nerve plexus and
supply terminal endings to the corneal epithelium. The posterior nerves supply fibres to the posterior stroma.\textsuperscript{40,41}

Fascinating observations on the pattern formed by the normal human sub-basal nerve plexus have been made by Patel and McGhee in the last ten years. Through \textit{in vivo} confocal microscopy, they have demonstrated that the nerve fiber bundles radiate towards a clockwise whorl or vortex pattern (whorl-like complex) with a centre located 1-2 mm below the corneal apex (see figure 1.2).\textsuperscript{42}

\textbf{Figure 1.2} A montage of \textit{in vivo} confocal microscopy images depicting the architecture of the human corneal sub-basal nerve plexus (scale bar = 400 µm)\textsuperscript{42}
1.5 Anatomical alterations in keratoconus

Keratoconus is a condition in which the cornea assumes a conical shape as a result of progressive, noninflammatory thinning of the stroma. It is the most common of the corneal ectatic disorders. As the cornea thins, it no longer maintains its shape, resulting in a protrusion of the apex, inducing irregular astigmatism, myopia, and marked reduction in the quality of the visual optics.\cite{43} The classic triad of histopathological findings in keratoconus consist of thinning of the corneal stroma, breaks in Bowman’s layer, and deposition of an iron ring in the basal epithelium (Fleischer ring).\cite{44} However, as the disease progresses, alterations in every layer of the cornea occurs, including downgrowth of the epithelium into Bowman’s layer, loss of keratocyte density, reduction in the number of collagen lamellae, and breaks in Descemet’s membrane and the underlying endothelium during acute corneal hydrops. A more in-depth analysis of the cornea in the diseased state can be found in Chapter 4.
Chapter 2

Physiology and Function
of the Cornea
2.1 Introduction

The primary functions of the cornea include not only the transmission of light but also the refraction of light in such a way as to allow the formation of a clear image on the fovea. In order to achieve these functions optimally, the cornea requires a smooth surface with a healthy tear film and epithelium, the maintenance of optical clarity, and the ability to respond adequately to trauma and wounding.

2.2 Tear film

The normal tear film is about 7-10 µm in thickness and 6-8 µl in volume. The tear film consists of three layers: 1) a superficial lipid layer (0.1 µm), which is produced by the Meibomian glands and the glands of Moll and Zeis; 2) an aqueous layer (7.0 µm), which is produced by the main and accessory lacrimal glands; and 3) a mucin layer (0.02 – 0.05 µm), which is produced by conjunctival goblet cells.

The lipid layer is unique in that it consists of a mixture of polar and neutral lipids with a melting point (35°C) in order to maintain a fluid layer on the ocular surface. The polar lipids are in contact with the aqueous layer of the tear film whereas the neutral lipids are at the air interface.

The aqueous component contains several proteins including immunoglobulin A, lactoferrin, G protein, tear-specific prealbumin, and lysozyme. The proteins are secreted in two ways: Constitutively, in which the proteins are immediately
released into the lacrimal gland acinar lumen upon production and not stored\(^\text{49}\) and by the Regulatory pathway, in which most proteins are stored in secretory granules within acinar cells until appropriate stimulus is generated.\(^\text{50}\) The majority of proteins are secreted by the regulatory pathway. Aqueous secretion by the lacrimal gland is under neural control and occurs in two stages.\(^\text{51}\) Firstly, acinar cells of the lacrimal gland secrete a fluid that has an electrolyte composition similar to that of plasma. Then, as this fluid passes through the duct system, the ductal cells modify it by secreting Potassium Chloride (KCl) via Na\(^+\)-K\(^+\)-ATPase pump. Water enters the secretory fluid using water channels known as aquaporins (figure 2.1).\(^\text{52}\)

The corneal epithelium is a minor contributor to the aqueous layer of the tear film, and secretes primarily electrolytes, such as sodium (Na\(^+\)) and chloride (Cl\(^-\)), and water, into tears.\(^\text{53}\)

Mucins produced by the conjunctival goblet cells are a heterogenous collection of high-molecular weight glycoproteins composed of a protein backbone with side chains of variable numbers of carbohydrates.\(^\text{54}\) The mucins are stored in secretory granules within the goblet cells until an appropriate signal is received for their release. Secretion is activated by stimulation of the parasympathetic and sympathetic nerves around the goblet cells.\(^\text{55}\) Upon stimulation, all the secretory granules are released in an explosive fashion as the mucin rapidly hydrates with the water of the tear film.\(^\text{50}\)

The tear film has several important functions.\(^\text{56}\)
1) It contributes to the smooth optical properties of the corneal surface.
2) It is the primary source of oxygen to the avascular cornea.\textsuperscript{57,58}
3) It provides lubrication between the eyelids and ocular surface.\textsuperscript{59}
4) It assists in the removal of foreign bodies, debris and exfoliated cells.\textsuperscript{59}
5) It contains antibacterial proteins that protect the ocular surface from bacterial infection.\textsuperscript{60}

**Figure 2.1** Diagram of lacrimal gland acinar cell showing the mechanism of electrolyte and water secretion. Possible roles of Calcium (Ca\textsuperscript{2+}) and protein kinase A (PKA) in activating ion movements are also indicated.\textsuperscript{48}

*Dashed arrow*, passive ion movements; *solid arrows*, active ionic movements.
2.3 Maintenance of the corneal epithelium and its response to wounding

The primary function of the corneal epithelium is to act as a barrier – not only from entry of foreign material externally but also from excess fluid entry into the stroma. Therefore it has unique regenerative and reparative properties that enable it to act as a successful barrier.

As described in Chapter 1, the corneal epithelium is under a state of continuous renewal with complete turnover of cells every 5-7 days. This occurs as the superficial cells are shed and the basal cells proliferate. Mitosis is limited to the basal epithelial cells at a rate of approximately 10-15% per day.\textsuperscript{8,61,62} New basal epithelial cells originate from limbal stem cells and migrate in a centripetal fashion into the centre of the cornea.\textsuperscript{63}

Following epithelial abrasion, mitosis ceases and the cells at the wound edge retract, thicken, and lose their hemidesmosomal attachments to the basement membrane.\textsuperscript{64} The cells expand to form an epithelial sheet, and the defect is closed by cell migration. The edges of the cell membranes ruffle and send out filopodia and lamellipodia toward the center of the wound.\textsuperscript{65} Cell migration is in an amoeboid manner, and begins within 5-6 hours of injury and progresses at a constant rate of 60-80 µm/hour until the entire defect is covered.\textsuperscript{66,67} After wound closure, mitosis resumes to restore the epithelium to its normal configuration. The cornea initially swells as a result of uptake of fluid from the tears. Once adhesions complexes are restored in the basal cells as the barrier is reestablished, the
cornea returns to normal thickness. The cellular events involved in the regulation of cell migration and the control of mitosis are hypothesised to be under autocrine and paracrine control involving peptide growth factors (e.g., epidermal growth factor, EGF). Proteolytic enzymes are also implicated including urokinase-type plasminogen activator and matrix metalloproteinases.

2.4 Corneal transparency and stromal function

Aside from the barrier function of the epithelium, the cornea also relies on several characteristics of the stroma to maintain transparency: 1) the specific dimensions and arrangement of the collagen fibres in the stroma; 2) the degree of stromal hydration; 3) the maintenance of the first two factors by the proteoglycans in the stromal extracellular matrix (ECM).

Stromal collagen possesses several unique characteristics in order to maintain corneal transparency. Firstly, the collagen fibres are weak scatterers of light because they vary only slightly in their diameter, and the variation in diameter is only a fraction of the wavelength of visible light. Secondly, the distribution of the collagen fibres is relatively uniform, with the distance between fibrils being less than half the wavelength of visible light. Stromal proteoglycans have a critical role in maintaining this structural uniformity. Multiple studies have demonstrated that the primary proteoglycan responsible in the cornea is lumican, and without lumican, an increase in collagen fibril diameter and a loss of the uniform spacing results, leading ultimately to a loss of corneal transparency.
The control of stromal hydration is also essential for transparency. The stroma is the most hydrated tissue in the body (78% water) with approximately 3.5 g H₂O/g dry weight. This hydrated state is maintained by the relative resistance of the stroma to diffusion of electrolytes compared to the epithelium and endothelium: 2000:1:10 (epithelium:stroma:endothelium). In actual fact the stroma is able to absorb even more water; this is called the imbibition pressure and is due to the water-binding capacity of the proteoglycans in the ECM. The ionic concentrations of sodium (Na⁺) and potassium (K⁺) in the stroma are higher than in the aqueous humor. However, in the stroma the ionic activity is less than in the aqueous due to cationic binding by the anionic sites on stromal glycosaminoglycans (GAGs), thereby resulting in lower osmotic and diffusional gradients. Water evaporation from the corneal surface also helps to reduce the tendency to swell. Evaporation occurs at a rate of 2.5 µl/cm²/hr and accounts for 5% of thinning throughout the day. Nonetheless, the main reason the stromal is kept from its natural tendency to swell is due to the metabolic pump function of the endothelium (see later). When one of these functions is lost, corneal swelling occurs, resulting in increased distance between collagen fibrils, and opacity ensues.

2.5 Stromal wound healing

Following wounding, keratocytes migrate, proliferate and undergo transformation into fibroblasts and myofibroblasts. These activated keratocytes synthesise collagen and GAGs.
It may take up to four years for the corneal stroma to regain tensile strength following trauma. Wounds near the centre of the avascular cornea heal slower than ones near the limbus or in neovascularised corneas.\textsuperscript{86} Due to the loss of specialised arrangement of the collage fibrils during healing, localised corneal opacity may ensue. This opacity may be temporary if the wound is small and transparency can be restored by the production of normal matrix components.\textsuperscript{47}

### 2.6 Endothelial physiology

As mentioned earlier, the most important reason the stromal water content is kept at 78% and not higher is due to the pump function of the endothelium. It is important for a continuous movement of fluid into the cornea to occur. As the cornea is avascular, this fluid serves as a source of nutrients including glucose and amino acids.\textsuperscript{26} However, it also needs to be removed continuously to prevent corneal swelling. Interestingly, it does so not through a water pump but a metabolic pump - the endothelial cell membranes contain active transporters for ions, amino acids, and sugars, and water moves down these osmotic gradients.
Figure 2.2 Model of ion and water transport across the corneal endothelium.

Activity of the metabolic pump sets up the osmotic gradient, resulting in movement of fluid from the stroma to the aqueous humor balancing the leak of fluid from the aqueous humor into the stroma.\(^{87,88}\)

The corneal stroma has a Na\(^+\) concentration of 160 mEq/L, however, only 134 mEq/L is osmotically active as the remainder is bound to stromal proteoglycans. Sodium ion concentration in the aqueous is 143 mEq/L.\(^{89}\) This produces an osmotic gradient of 163.8 mmHg drawing water out of the cornea.\(^{90}\) The Na\(^+\)-K\(^+\)-ATPase located in the basolateral membrane of the endothelial cell is critical for maintaining this gradient (figure 2.2). In a normal cornea there is on average 1.5 x \(10^6\) pump sites per cell.\(^{91}\)
Other important components of the metabolic pump include the \( \text{Na}^+ - \text{H}^+ \) exchanger that moves sodium into the endothelial cell and hydrogen out,\(^{87}\) as well as the bicarbonate (\( \text{HCO}_3^- \)) pump that transports bicarbonate from the cell into aqueous humor.\(^{92}\) Carbon dioxide (\( \text{CO}_2 \)) diffuses into the cells from the extracellular space. Carbonic anhydrase, present within the endothelium, catalyses carbon dioxide with water to form carbonic acid.\(^{93}\) Carbonic acid readily dissociates into hydrogen (\( \text{H}^+ \)) and bicarbonate (\( \text{HCO}_3^- \)) ions. These ions are then transported out of the cell by the \( \text{Na}^+ - \text{H}^+ \) exchanger, and the bicarbonate transporter, respectively. Carbon dioxide readily diffuses into the cell due to the acidity maintained in the extracellular fluid by the \( \text{Na}^+ - \text{H}^+ \) exchanger.

### 2.7 Endothelial wound healing

Human endothelial cells have no ability to replicate or self-repair following injury, therefore endothelial wound healing is largely dependent on the existing surrounding cells to expand and cover the defect. Therefore over time, a significant enlargement of endothelial cells can be seen in the aged cornea (polymegathism).\(^{94}\)

If the damage is extensive, endothelial cells can migrate to cover fill the defect. This can be up to distances of 250 µm from the wound edge.\(^{95}\) Subsequent remodeling of the cells back into its usual hexagonal shape follows. Restoration of endothelial barrier and pump function occurs once a confluent monolayer is re-established, returning the cornea to normal thickness.\(^{96}\)
Chapter 3

*Measuring Intraocular Pressure using Contemporary Corneal-based Techniques*
3.1 Introduction

Intraocular pressure (IOP) is the result of the balance of aqueous humor production by the ciliary body and outflow through the drainage angle. Its measurement is a fundamental part of every ophthalmic assessment. Normal intraocular pressure ranges from 10 – 21 mmHg. Deviations from this can result in significant visual morbidity in the form of hypotonous maculopathy if the IOP is too low, or glaucomatous optic neuropathy, the leading cause of irreversible blindness in the world today, if the IOP is elevated. IOP is also the method by which we monitor the effect of glaucoma treatment. Clearly, the importance of accurate IOP measurement cannot be over-emphasised.

This chapter reviews the current methods of IOP measurement.

3.2 Manometry

The current gold standard for IOP measurement is manometry. It is an invasive technique that measures true intraocular pressure, and is not influenced by characteristics of the external ocular surface. It is therefore is the reference standard by which all IOP measurements should be judged.

The technique utilizes a hollow needle that is inserted into the anterior chamber. The needle is connected to a reservoir calibrated in either centimetres of water (cmH₂O) or millimetres of mercury (mmHg) that displays IOP as aqueous flows out through the hollow needle into the reservoir. Aqueous does so because IOP is
normally higher than atmospheric pressure. Manometric measurements as such are accurate but impractical for clinical practice, and therefore primarily reserved for laboratory investigations.

Most of the commonly used tonometers of today have been validated and calibrated on human cadaver eyes against a manometric reference value.\textsuperscript{97-101}

\section*{3.3 Applanation tonometry}

Any form of applanation tonometry is based on the Imbert-Fick law: pressure = force/area. In lay terms, it dictates that the pressure inside a flexible sphere with thin walls can be closely approximated by knowing the force necessary to flatten (applanate) a given area of the sphere.

The Goldmann applanation tonometer (GAT; Haag-Streit, Bern, Switzerland) is based on this principle and is the current gold standard for non-invasive IOP measurement. It is also the most widely utilised tonometer by the ophthalmologist. The device requires attachment to a slit-lamp biomicroscope (figure 3.1). It has an applanating surface with a diameter of 3.06 mm placed in the center of a plastic cylinder. The plastic cylinder is attached to an arm pushed forward through a spring-loaded knob. The force applied to the cylinder can be read off a scale on the dial at the side of the instrument and is very finely tuned.

As the GAT requires direct applanation of the cornea, topical anesthetic and fluorescein dye are placed in the eye. The dye spreads over the corneal surface
when mixed with tears and, when activated by cobalt-blue light emitted by the slit-lamp, it fluoresces a brilliant yellowish-green. To determine the IOP, the plastic cylinder is applanated against the corneal surface. This action results in two mires of fluorescent yellow-green, vertically stacked. The mires represent the ring of tear meniscus formed by the applanating surface that has been refracted by the two prisms within the plastic cylinder. The orientation and power of the prisms are such that the two mires are optically separated by exactly 3.06 mm. The force dial is turned until the applanated area is exactly 3.06 mm in diameter, and this is achieved when the inside edges of the end of each split ring just touch.\textsuperscript{102}

**Figure 3.1** Demonstration of the Goldmann applanation tonometer.

Since the idea of a fixed-area applanation tonometer first arose in 1888 with
Fick,\textsuperscript{103} and later developed into the tonometer that we know today by Goldmann,\textsuperscript{97} its accuracy, repeatability and reliability have been well-tested. Studies have shown that intra-observer reliability of GAT is 1.7 mm Hg, and inter-observer variability is 0.4 mm Hg.\textsuperscript{104} It also comes in a portable form (Perkins applanation tonometer) that allows the same accuracy as the slit-lamp mounted version but with the advantage of being able to examine the patient in any position.\textsuperscript{105}

The Imbert-Fick law is applicable to surfaces that are perfectly spherical, elastic and infinitely thin. However, the cornea has a finite thickness and the globe is not a perfectly elastic structure. Therefore corneal properties such as significant corneal astigmatism (>3 dioptres),\textsuperscript{106} corneal thickness,\textsuperscript{107-109} corneal curvature,\textsuperscript{110} and corneal biomechanics\textsuperscript{111,112} can influence GAT values. It tends to over-estimate IOP in thick corneas, and under-estimate in thin corneas. Due to this deficiency, the GAT is not an ideal tonometer in eyes with an abnormal cornea. It can also be altered by variations in fluorescein and tear film.\textsuperscript{101} Other disadvantages include the need for topical anaesthesia, and the possibility of transmission of infection if not sterilised properly between patients.

3.4 Non-contact air-puff tonometry

The non-contact air-puff tonometer works on the same basic principle as the GAT, except that it usually performs electronically. A puff of air is emitted onto the cornea by an air puff generator. The force of the air stream increases linearly over several milliseconds and progressively flattens the cornea to produce a concavity.
The air puff is designed so that it hits the cornea with a known and reproducible area. An optical sensor detects an oblique light, which is reflected by the cornea when it reaches a specific concavity. An immediate signal is then sent by the sensor to turn off the air pulse generator. The IOP is determined by computer calculations of the force of the air puff and the known area and displayed digitally.

Good correlation has been described by most studies comparing the non-contact tonometer with the GAT. Unlike the GAT, the non-contact air-puff tonometer is not influenced by variations in tear film. There is also no need for topical anaesthesia or fluorescein application. These factors, and its ease of application, make the non-contact tonometer a very popular screening tool, particularly in the optometric setting.

However, similar to the GAT, it is influenced by corneal thickness. There is a positive correlation between the thickness of the cornea and IOP value. The magnitude of this correlation increases with thicker corneas.

3.5 Combination applanation-indentation tonometry

The TonoPen (Reichert, Inc., Depew, NY, USA) is based on the design of the MacKay-Marg tonometer, which is no longer in production. It operates on the principles of both applanation and indentation. It is a handheld, battery-operated instrument (figure 3.2). The tip is a microplunger that is connected to a sensitive
transducer, which converts plunger displacement into an electrical waveform much like an electrocardiogram. To reduce infection risk, the tip is covered by a disposable latex cover and applied perpendicularly to gently indent an anaesthetised cornea. The IOP is processed from several applanations. An acceptable applanation is indicated by an audible click after contact with the cornea. A microprocessor averages several acceptable waveforms to provide a digital readout of IOP on a liquid crystal display, with an estimate of the variability between the component readings.

**Figure 3.2 The TonoPen**

There is generally good correlation between the TonoPen and either manometry or applanation tonometry in normal physiologic IOP range. However, for lower readings, the TonoPen tends to over-estimate, and conversely for higher readings, compared to the GAT. The overall agreement between the measurements of the two instruments is good but a small percentage of large difference (≥ ±5 mm Hg in 7.4%) may be of concern in a population-based survey.

The TonoPen is especially useful for obtaining IOP from scarred, edematous,
irregular, or transplanted corneas as it is less dependent on corneal rigidity and elasticity and the endpoint is reached electromechanically, not optically.\textsuperscript{130-132} It can measure IOP through a bandage contact lens and with the patient in any position.

### 3.6 Rebound tonometry

The principle of rebound tonometry was first introduced in 1931 by Obbink.\textsuperscript{133} He introduced the idea of a handheld ballistic device that detected the return-bounce motion of an object hitting the cornea. Subsequent devices have been introduced but without great success due to deficiencies in application.\textsuperscript{134} The iCare rebound tonometer (ICT; Icare Finland Oy, Helsinki, Finland) was first introduced in 1997 and validated on mouse eyes.\textsuperscript{135-138}

In iCare rebound tonometry, a solenoid ejects a magnetized probe onto the cornea. The same solenoid detects the impact of the probe when colliding with the eye and the motion of the bounce back. A microprocessor analyses the deceleration of the probe following impact, which is less at low IOP than high IOP; therefore, the higher the IOP, the shorter the duration of impact.\textsuperscript{102} The measurement is taken by placing the adjustable rest on the patient’s forehead. Measurement of IOP with rebound tonometry does not require topical anesthesia due to the short duration and low impact of the contact. It also has minimal infection risk due to the design of disposable probes.
The iCare has been found to slightly over-estimate IOP compared to the GAT in healthy eyes.\textsuperscript{139} It has, however, been found to be comparable to the TonoPen.\textsuperscript{140,141}

Like applanation tonometers, it positively correlates with corneal thickness,\textsuperscript{142} and on thick corneas, the iCare over-estimates the IOP even more than the GAT and TonoPen.\textsuperscript{143,144}
Its advantage is that it consistently rates highly by patients in terms of comfort. This and its ease of use make it a good instrument for screening purposes, especially for use by non-ophthalmic personnel.

### 3.7 Contour tonometry

Pressure is defined as freely-relocatable molecules in liquids and gases as a uniform force distribution acting perpendicular to all boundaries (Law of hydrostatic pressure by Blaise Pascal [1623–1662]). When non-invasive IOP measurement is conducted, this law is implied and we assume that the generation of forces outside the cornea corresponds to forces generated by the intracameral pressure.

What is unique about dynamic contour tonometry is a tonometer tip that has a specifically-formulated contour. It operates on the theory that a hypothetical corneal shape (contour) will form when the pressure on either side of the cornea is equal. The force distribution that is needed to gently fit the corneal surface to that hypothetical contour counterbalances the force distribution generated by the intraocular pressure.

Dynamic contour tonometry also allows simultaneous measurement of ocular pulse amplitude (OPA). The OPA is an indirect indicator of choroidal perfusion and reflects the ocular blood flow corresponding to the heart pulse as a function of time. The OPA may be of significant relevance in the clinical course of
Pascal Dynamic Contour tonometer (PDCT; Swiss Microtechnology AG, Port, Switzerland) is the practical implementation of dynamic contour tonometry. It is a digital, slit-lamp mounted instrument that handles similarly to the GAT. It consists of a transparent pressure-sensing tip with a contoured contact. The piezo-electric pressure sensor contained within the tip not only generates an electric signal that is proportional to the IOP, but also an audio signal, which indicates the quality of contact between the tip and the cornea. The pressure signal is modulated by the pulsatile ocular blood flow. A good measurement requires approximately five seconds of contact and is terminated by removing the tonometer head from the cornea. Due to the duration of contact, application of topical anaesthesia is required.

To avoid spread of infection and to protect the delicate pressure sensor in the tonometer tip, disposable SenseCaps are necessary to cover the tonometer tip and must be replaced after each patient use.

The IOP is generated by the software and displayed on a LCD screen. The OPA is derived from IOP modulations caused by cardiac pulsations. Additionally, a quality score is also given that provides an indication of the reliability of the results. The quality score ranges from Q1 (good measurement) to Q5 (bad measurement). It is based on the number of valid data points, noise level, presence of artifacts, and regularity and shape of pulsations.
It is important to note that PCDT provides the diastolic IOP, whereas GAT IOP is an estimate of mire pulsations (i.e. a mean of systolic and diastolic IOP). The OPA is the difference between systolic and diastolic IOP. Another feature of PDCT is the provision of an indication of the range of pressures the optic nerve head is exposed to over time. This pressure curve can be printed out on an optional remote infrared-activated printer.

Most studies assessing the PDCT analysed its association with CCT in comparison with GAT. In the majority of these studies, PDCT has been shown to provide higher IOP values than GAT.\textsuperscript{104,151-159} While most studies have found a positive correlation between CCT and GAT,\textsuperscript{98,110,152-155,159-161} a few have found a weakly positive correlation between GAT and CCT in normal eyes but not glaucomatous eyes.\textsuperscript{158,162} In contrast, the majority of studies found no association between PDCT and CCT.\textsuperscript{153-157,159-163} Additionally, the PDCT has been found to be less influenced by other corneal factors such as age,\textsuperscript{104} and alterations post-corneal refractive surgery.\textsuperscript{164,165} Despite being a relatively new device, it has harnessed tremendous popularity as the tonometer of choice.

Its relative disadvantage is its handling. Because the measurement takes at least 5 seconds to obtain, patient cooperation is paramount to achieve a reliable result. Repeated measurements are often necessary to obtain a score of Q1-3. Some patients may not be suitable, as they cannot maintain steady eye contact and head position necessary for the contour matching.
3.8 Ocular Response Analyser

While significant research has been devoted to the influence of corneal thickness on IOP values measured by applanation tonometry, there is substantial evidence that other corneal properties affect applanation tonometric values, and may be more relevant than central corneal thickness. Liu and Roberts\textsuperscript{112} used a mathematical model to quantitatively analyse the effect of individual corneal biomechanical parameters – such as thickness, radius of curvature and modulus of elasticity - on IOP obtained by applanation tonometry, and found that variations of the elasticity of the cornea within a range predicted to occur within a normal population could result in an error of IOP measurement as high as 17 mmHg.

The Ocular Response Analyzer (ORA; Reichert Inc, Depew, NY) is a new instrument that proposes to account for such corneal biomechanical variations. It is an air- puff tonometer that ejects 20 ms of air impulse and monitors the time course changes of the cornea. The air puff deforms the cornea into a slight concavity, and an electro-optical collimation detector system monitors the pressures at which the cornea flattens inward and outward.\textsuperscript{166} The availability of high-speed imaging instruments has enabled the capture of cross-sections of the cornea in real-time during an air puff event.

The ORA provides two different IOP readings. The Goldmann-correlated IOP (IOPg) is the mean between the inward and outward applanation pressures; the difference between these two pressures allows the ORA to compute two corneal properties: 1) corneal hysteresis (CH) is thought to represent the viscoelastic
properties of the cornea; 2) the corneal resistance factor (CRF) is thought to predominantly reflect the elastic resistance of the cornea, and could reflect the overall resistance of the eye.\textsuperscript{166,167} The second IOP reading is the corneal-compensated IOP (IOPcc), which is derived from the difference between the 2 appplanation pressures using the formula $P_2 - kP_1$, where $P_1$ and $P_2$ are the first and second appplanation pressures, respectively, and $k$ is a constant.\textsuperscript{110} As the difference between $P_1$ and $P_2$ is related to the corneal biomechanical properties, the value of IOPcc is supposed to represent a measure of intraocular pressure that is free of corneal influence. The constant $k$ has a value of 0.43, which was derived from a study on intraocular pressure changes before and after corneal refractive surgery. (D. Luce, PhD, Reichert Inc, written communication, September 2005).

Highly consistent inter- and intra-observer repeatability, as well as inter- and intra-sessional repeatability, has been demonstrated on the ORA.\textsuperscript{168-171}

Medeiros and Weinreb\textsuperscript{110} assessed the relationship between corneal biomechanical properties and IOP as measured by the GAT and ORA in 153 normal eyes of 78 subjects, and found that on multivariate analysis, GAT-measured IOP was significantly associated with CCT, whereas the IOPcc were not associated with any of the independent variables, including CCT, axial length, corneal curvatures, or age.
However, if the ORA corneal resistance factor (CRF) was incorporated into the multiple regression model, then only the CRF was found to be significantly associated with the GAT IOP, and not CCT, corneal curvature, axial length, or age. Furthermore, a positive correlation was observed between CRF and CCT and between CRF and corneal curvature. The authors therefore postulated that CRF is not solely measure of corneal material properties, but is rather an index that aggregates the effects of CCT, tissue material properties, and corneal curvature.

They also directly compared IOP values measured by the ORA with GAT, and found no statistical difference between GAT IOP and IOPcc. The magnitude of IOP did not influence the difference between the two measurements. However, the difference between the two measurements was affected by CCT: each 100-μm increase in corneal thickness resulted in 2.256 mm Hg increase in the difference.
GAT IOP-IOPcc (P = 0.005).\textsuperscript{110}

The consistency between GAT IOP and IOPcc has not been found across all studies. Moreno-Montanes \textit{et al.}\textsuperscript{170} assessed 262 eyes in 262 subjects, including those with and without primary open angle glaucoma, and found the GAT consistently measured higher IOP than the ORA IOPg and IOPcc. Furthermore, there was consistently a difference IOPg and IOPcc, not only in the glaucomatous eyes but also in the normal eyes. The study did not attempt to account for the difference through other corneal parameters and therefore the difference is unexplained.

The ORA is influenced by tear film. A dry cornea can lead to falsely high CH.\textsuperscript{172} Single measurements should be made quickly (within 20 ms) in order to avoid alterations of the tear film layer resulting from reflections of the infrared light.\textsuperscript{172}

Many studies have been conducted assessing the validity of the ORA measurements of CH and CRF but as this chapter is primarily concerned with methods of IOP measurement, they will not be included.

The ORA shows promise in providing IOP values that are less influenced by corneal properties than GAT. However, as it is a relatively new device, larger prospective, blinded studies, ideally with manometric validation, are required.
References for Section I


96. Yee RW, Geroski DH, Matsuda M, Champeau EJ, Meyer LA, Edelhauser HF. Correlation of corneal endothelial pump site density, barrier function,


133. Obbink J. *Onderzoek naar het verband tusschen inwendigen oogdruk en balistische reacties*. The Netherlands, The University of Utrecht; 1931.


141. van der Jagt LH, Jansonius NM. Three portable tonometers, the TGDc-01, the ICARE and the Tonopen XL, compared with each other and with Goldmann applanation tonometry*. Ophthalmic Physiol Opt 2005;25(5):429-435.


156. Punjabi OS, Ho HK, Kniestedt C, Bostrom AG, Stamper RL, Lin SC. Intraocular pressure and ocular pulse amplitude comparisons in different


171. Sporl E, Terai N, Haustein M, Bohm AG, Raiskup-Wolf F, Pillunat LE.  
[Biomechanical condition of the cornea as a new indicator for pathological  
and structural changes]. *Der Ophthalmologe : Zeitschrift der Deutschen  

172. Terai N, Raiskup F, Haustein M, Pillunat LE, Spoerl E. Identification of  
biomechanical properties of the cornea: the ocular response analyzer. *Curr  
Section II

Studies on keratoconus and acute corneal hydrops
Chapter 4

Acute corneal hydrops in keratoconus –

new perspectives
4.1 Abstract

Purpose
To summarise the current concepts and recent literature regarding the epidemiology, pathogenesis, imaging modalities and treatment of acute hydrops in keratoconus.

Methods
Review and synthesis of selected literature, with interpretation and perspective.

Results
Acute corneal hydrops is an incompletely-understood complication of keratoconus, characterised by the development of marked corneal oedema caused by a break in Descemet's membrane, allowing aqueous to enter the corneal stroma and epithelium. Although acute hydrops is usually self-limiting and clinical signs of oedema typically resolve after two to four months, it often leaves a vision-impairing scar, necessitating and expediting the need for corneal transplantation. Studies have identified risk factors that lead to acute hydrops. Modern imaging modalities such as ultrasound biomicroscopy, anterior segment optical coherence tomography, and in vivo confocal microscopy have enlightened us to the microstructural changes that take place during acute hydrops, the factors that influence its duration and sequelae. Newer treatment regimens not only have seen a reduction in the duration of corneal oedema during acute hydrops, but have improved the survival of corneal grafts after transplantation for resolved hydrops.
Conclusions:

Effective management of acute corneal hydrops in keratoconus is based on recognising and addressing the risk factors, treating the acute event effectively and promptly to reduce the duration of oedema and its complications, and ultimately successful corneal transplantation with acceptable long-term graft survival rates. Improved in vivo imaging of the cornea during acute hydrops has led to an enhanced understanding of the pathogenesis and ultrastructural changes of the condition, and in turn has resulted in improved management of the disease.
4.2 Introduction

Keratoconus is an ectatic corneal disorder, classically described as progressive, non-inflammatory and characterised by central corneal thinning, protrusion and irregular myopic astigmatism. Although originally described in detail in 1854 by Dr John Nottingham, the substantial portion of our understanding of the condition has only been acquired in the last 50 years. Nonetheless, the earliest report of corneal oedema related to keratoconus dates to a description by Terrien in 1906, however, the specific term “acute corneal hydrops” (hydrops corneae) was only popularised in 1940 following animal modelling and detailed clinical descriptions of focal disruption of Descemet’s membrane. Acute corneal hydrops remains an incompletely understood complication of keratoconus. It can also occur in other corneal ectasias, reportedly with higher frequency in pellucid marginal corneal degeneration (PMCD) (6-11%) and keratoglobus (11%). Additionally it has been reported as a very rare late complication following penetrating keratoplasty for keratoconus.

4.3 Pathogenesis

Acute hydrops is the development of marked corneal oedema caused by a break in Descemet’s membrane and endothelium, allowing aqueous to enter the corneal stroma and epithelium (figure 4.1). The elasticity of Descemet’s membrane means it retracts or coils when it breaks under tension. Basu et al. postulated that exact re-approximation of the displaced margins, either spontaneously or with C3F8, is not possible and resolution of corneal hydrops probably involves two
steps. Firstly, the detached Descemet's membrane has to reattach to the posterior stroma, the time for this depends on the depth of the Descemet's membrane detachment. Secondly, the endothelium has to migrate over the gap between the two broken edges of Descemet's membrane, the interval for this depends on the dimensions of the Descemet's membrane break. Thus, insertion of $\text{C}_3\text{F}_8$ can hasten the first step but not the second.

**Figure 4.1** Anterior segment photograph demonstrating severe acute corneal hydrops in a patient with keratoconus. Extensive corneal oedema with epithelial bullae is visible and a vertical defect in Descemet's membrane is present in the central cornea
Although acute hydrops is usually self-limiting and clinical signs of oedema typically resolve after two to four months,\textsuperscript{9,10} it often leaves a vision-impairing scar (figure 4.2) necessitating and expediting the need for corneal transplantation. In some cases, corneal neovascularisation may occur (figure 4.2, arrow) and this has significant implications on the patient’s future management and prognosis. Previous studies suggest that oedema near the limbus and intrastromal cleft formation in cases of acute corneal hydrops may be considered risk factors for stromal neovascularisation. Any associated inflammatory response, which may be greater in patients with atopy, has also been suggested as a potential stimulus to neovascularisation.\textsuperscript{11}

**Figure 4.2** Anterior segment photographs of a resolved case of acute corneal hydrops in a patient with keratoconus. a. A large central stromal scar and peripheral corneal neovascularisation (arrow) are demonstrated on diffuse illumination. b. Retro-illumination highlights a large break in Descemet’s membrane (arrow heads).
4.4 Epidemiology and risk factors

The prevalence of keratoconus has been reported to vary in different studies internationally, from 8.8 to 54.4 per 100 000.\textsuperscript{12} Corneal hydrops is relatively uncommon and is estimated to occur in 2.6 – 2.8\% of patients with keratoconus.\textsuperscript{13,14} Interestingly, the mean age of onset of corneal hydrops is similar across studies, typically around 25 years of age, with a male preponderance.\textsuperscript{13,15}

Although an ethnic variation in prevalence of keratoconus is well established, few studies have specifically identified ethnic associations with the development of acute hydrops in keratoconus. In a recent study from New Zealand, where keratoconus is the most common indication for corneal transplantation,\textsuperscript{16} the authors noted that Pacific ethnicity is strongly associated with the development of acute hydrops, whereas, New Zealand European ethnicity is negatively associated with hydrops development.\textsuperscript{15}

Whilst other risk factors have been identified in several studies, due to the relatively small numbers of subjects in most studies, it is difficult to isolate the risk factors of hydrops from those risk factors for keratoconus \textit{per se}. However, two retrospective reviews attempted to determine the clinical factors associated with the development of acute hydrops, and each study included over 100 patients. Tuft \textit{et al.}\textsuperscript{13} identified earlier age of diagnosis, steeper keratometry and poorer Snellen visual acuity at the time of diagnosis of keratoconus to be strongly associated with subsequent development of corneal hydrops. Corneal hydrops also developed at a greater rate in eyes with severe allergic eye disease. Fan
Gaskin et al.\textsuperscript{15} also identified poorer visual acuity at first presentation to the tertiary referral centre for keratoconus to be associated with subsequent hydrops development. A history of eye-rubbing was also associated with hydrops development - independent of a history of atopy and contact lens wear (which were not associated with greater likelihood of hydrops). Somewhat paradoxically, subjects who develop hydrops were found to be less likely to have a family history of keratoconus in this study.\textsuperscript{15}

4.5 Natural history of corneal hydrops

After the rupture of Descemet’s membrane, it may retract and curl anteriorly to form scrolls, ridges or strands around attached fragments of stroma.\textsuperscript{7} This is thought to be the reason why acute hydrops takes longer to resolve than localised corneal oedema caused by a breach of Descemet’s membrane during cataract surgery on a keratoconic eye.\textsuperscript{17}

The onset of acute hydrops is usually heralded by marked epiphora, followed by intense photophobia and pain, associated with markedly reduced visual acuity. Most cases of acute corneal hydrops resolve spontaneously over 2-4 months\textsuperscript{9,10} as the adjacent endothelial cells enlarge and migrate to cover the defect.\textsuperscript{18} Secondary flattening of the cornea may facilitate improved contact lens fitting, but central corneal scarring typically mandates corneal transplantation to restore visual function.
Unsurprisingly, greater area of corneal involvement by hydrops corresponds to a longer duration for the oedema to resolve, increased risk of neovascularisation, and ultimately a poorer visual outcome.\textsuperscript{19} Other complications of acute hydrops include infection, pseudocyst formation, malignant glaucoma, and corneal perforation.\textsuperscript{4,13} A history of hydrops may also predispose patients to greater likelihood of episodes of endothelial graft rejection after penetrating keratoplasty.\textsuperscript{13,20}

### 4.6 Imaging of corneal hydrops

Traditional modalities of anterior segment imaging in corneal ectasia can be problematic in acute hydrops due to the difficulty of imaging through an oedematous cornea. The advent of ultrasound biomicroscopy (UBM), anterior segment optical coherence tomography (AS-OCT) (figure 4.3), and \textit{in vivo} confocal microscopy (IVCM) (figure 4.4) has revealed some of the ultrastructural changes that occur during acute hydrops \textit{in vivo}.\textsuperscript{8,10,17,21} Previously such ultrastructural changes could only be extrapolated from \textit{ex vivo} observations on “resolved” tissue - obtained following corneal transplantation. These new technologies have also improved our ability to predict duration of oedema, likelihood of neovascularisation and monitor response to therapy.\textsuperscript{8,9,17,21}

An ultrasound biomicroscopy study has confirmed that intrastromal oedema is directly related to rupture of Descemet’s membrane. Direct visualisation of Descemet’s tear revealed areas of deficiency under the location of maximum stromal oedema.\textsuperscript{21} In the same study, following intracameral $C_3F_8$ at week zero,
ultrasound biomicroscopy demonstrated a complete unrolling and re-apposition of Descemet’s membrane to the corneal stroma by three weeks, with complete resolution of oedema in 92.3% of cases by six weeks.

**Figure 4.3** Anterior segment optical coherence tomography image of a resolved case of acute corneal hydrops in a patient with keratoconus. Central corneal stromal scarring and a focal detachment of Descemet’s membrane are demonstrated.

In a similar study, Nakagawa *et al.* assessed a series of thirteen consecutive hydrops corneas with ultrasound biomicroscopy and discovered not only was rupture of Descemet’s membrane identified in all eyes, but all eyes also exhibited intrastromal clefts. In eleven of thirteen eyes the clefts were connected with the anterior chamber on the ultrasound biomicroscopy images. The authors hypothesised that severe corneal oedema results from the presence of intrastromal clefts by increasing the surface area exposed to the anterior chamber. The gap between Descemet’s membrane and the stroma may also delay the closure of Descemet’s membrane and ultimately the resolution of corneal oedema.
Basu et al.\textsuperscript{8} examined 24 eyes with acute hydrops with anterior segment optical coherence tomography and published a retrospective study assessing the serial observations throughout the duration of hydrops. Interestingly, they identified three patterns of Descemet’s membrane appearance during acute hydrops on anterior segment optical coherence tomography: detachment with break and rolled ends, detachment with break and flat ends, and detachment with no break. However, they acknowledged that because anterior segment optical coherence tomography scans are taken at 45° intervals, a small planar break could have been missed, as detachment without break would question our present understanding of the mechanism of hydrops development. This study revealed that, without intervention, not only is the duration of oedema affected by the size of Descemet’s membrane break, but also the depth of the Descemet’s membrane detachment. The third factor to influence duration of clinical oedema is intervention with intracameral C\textsubscript{3}F\textsubscript{8} (see next section).

\textit{In vivo} confocal microscopy is a technology that has revolutionised imaging of the cornea in recent years. Lockington et al. conducted a prospective study of acute hydrops in keratoconus as assessed by \textit{in vivo} confocal microscopy.\textsuperscript{10} This study revealed the presence of presumed inflammatory cells, in four of the ten eyes studied, and postulated these may be associated with neovascularisation. These cells were hyper-reflective with round cell bodies (figure 4.4, left), present in the epithelium and anterior to mid-stroma. In two eyes, these cells persisted throughout the duration of hydrops, beyond clinical resolution. In these two cases, other unique cellular structures were also identified: elongating cells with small cell bodies were noted in the anterior stroma at 2-3 months (figure 4.4, middle) and at
3-months after presentation, both of these corneas also exhibited unusual stromal cells with large, round, speckled cell bodies and elongated cells with branching cell processes (figure 4.4 right). Both corneas developed stromal neovascularisation and were the only corneas to do so during the study.

**Figure 4.4**  *In vivo* confocal microscopic images demonstrating cellular changes observed during the course of acute corneal hydrops in patients with keratoconus. Hyper-reflective cells with small, round cell bodies (presumed to be inflammatory cells) may be observed in the anterior stroma at the time of presentation (left). Elongated cells with small cell bodies observed in the anterior stroma 2-3 months after the onset of severe acute hydrops (middle). Stromal cells with large, round speckled cell bodies and elongated cells with branching cell processes observed 3-months after the onset of severe acute hydrops in an eye that had developed corneal neovascularisation (right).

Contemporary imaging modalities have enabled significantly greater insight into the pathophysiology of corneal hydrops. However, in a clinical setting, UBM and IVCM require more technical skills and operator experience. Notably, although the latter has increased our understanding of hydrops at the microstructural level,
currently IVCM has a minimal role in management. In contrast, AS-OCT is easier to capture than UBM or IVCM and therefore might more readily assist in ascertaining whether certain interventions may be beneficial (see next section).

4.7 Treatment of corneal hydrops

Historically a variety of methods have been utilised to treat acute corneal hydrops, including the excision of a vertical ellipse of cornea, a conjunctival flap, medial tarsorrhaphy, paracentesis, ocular hypotensive treatment, chemical or thermal cauterisation of the cornea, injection of autologous blood into the anterior chamber, and emergency penetrating keratoplasty. However, advocacy of some earlier treatments was based on a lack of understanding of the natural history of hydrops.

Acute hydrops is a condition that generally resolves without intervention over 2-4 months, during which time the sight and comfort of the patient is significantly compromised. Longer duration of oedema is also more likely to lead to complications such as neovascularisation. Therefore many therapeutic options are aimed at safely facilitating speedier recovery. Other forms of treatment are targeted at minimising or eliminating complications of hydrops.

Treatment regimens can be divided into conservative, medical and surgical options:
Most conservative treatment includes observation and topical lubrication for comfort. Pressure patching and bandage contact lens have also been advocated to reduce oedema.\textsuperscript{4}

Medical therapy usually encompasses topical hypertonic saline (5\%) to reduce intra-stromal oedema, topical corticosteroids to reduce inflammation and prevent neovascularisation, and cycloplegic agents to reduce pain.\textsuperscript{4} Unfortunately, the evidence behind any of these regimens remains limited, case-based and largely anecdotal.

Theoretically, topical corticosteroids may reduce the risk of corneal neovascularization or lessen the extent of progression should neovascularization occur. However, there is little evidence in the literature to support this theory. Indeed, although widely used in clinical practice, some studies have found topical corticosteroids to be entirely ineffective in arresting the progression of stromal neovascularization in corneal hydrops.\textsuperscript{11}

As the clinical indications for use of anti-vascular endothelial growth factor (anti-VEGF) agents in corneal diseases increase, future studies may investigate their potential role in the management of intractable neovascularisation after acute corneal hydrops.

In the last 10 years, intracameral injection of air or expansile gas has been advocated as a treatment for acute hydrops.\textsuperscript{23-27} Proponents of this therapy advocate that the presence of air/gas in the anterior chamber encourages re-
apposition of Descemet’s membrane to the corneal stroma and thus promotes reattachment, thereby speeding up the resolution of stromal oedema. The air/gas also acts as a mechanical barrier, preventing further egress of aqueous humor into the stroma.\textsuperscript{23-25,27} The procedure involves the formation of paracentesis to inject air/gas with or without an accompanying surgical iridectomy/iridotomy to avoid pupil block glaucoma.

Miyata \textit{et al.} performed a retrospective study comparing the efficacy and safety of intracameral injection of air (0.1mL) with conventional medical therapy in the treatment of acute hydrops in keratoconus.\textsuperscript{23} Conventional therapy in this study included: no therapy, ofloxacin ointment and patching, sodium chloride eye drops (5%), or dexamethasone ointment (0.1%). They noted that those eyes that received intracameral air injections recovered more rapidly compared with those that did not. However, seven out of the nine eyes in the treatment group required two or more repeated injections of air. Patients were asked to remain supine for as long as possible following the injection of air and patients in the treatment group were also give Acetazolamide 750 mg per day for three days to prevent ocular hypertension, thus potentially confounding the possible mechanism for the faster recovery. The visual acuity following recovery was not superior in the group treated with air compared to conventional treatment.

In order to avoid repeated injections in clinical practice, other investigators have experimented with longer-lasting gases such as sulphur hexachloride (SF\textsubscript{6}) and perfluoropropane gas (C\textsubscript{3}F\textsubscript{8}).
Panda et al. investigated the use of sulfur hexafluoride (SF₆) in the management of corneal edema caused by acute corneal hydrops secondary to keratoconus. They compared intracameral injection of 0.1 mL of 20% SF₆ treatment in a prospective study, with stored data of patients previously treated with conventional therapy. Patients who received intracameral SF₆ were also administered 250 mg of Acetazolamide three times daily and asked to remain supine until the gas bubble was resorbed. Conventional treatment included g. sodium chloride (5%) every 4 hours, and twice-daily oc. sodium chloride (6%), g. tropicamide (1%) and g. ciprofloxacin (0.3%), until resolution of corneal edema and formation of corneal scar. There were nine patients in each group. Despite the use of SF₆ rather than air, two or more injections were still required in six of the nine patients. Corneal oedema began to resolve in the injection group at 3 weeks but did not begin to do so in the (historic) conventional group until 12 weeks. The 12-week best spectacle-corrected visual acuity (BSCVA) was correspondingly poorer in the conventional group (mean logMAR BSCVA: 0.39 in injection group and 0.24 in conventional group, P = 0.016).

Traditionally perfluoropropane has not been advocated for intracameral use as it is thought to be toxic to the corneal endothelium. However, its successful use has been reported for the reattachment of Descemet’s tear following complicated cataract surgery. Shah et al. subsequently reported a single case report of rapid resolution of oedema following two injections of intracameral C₃F₈. The first injection was 0.1 ml of 10% non-expansile concentration of C₃F₈. However, after one-week the gas bubble had significantly reduced in size and the oedema had not improved. Therefore a second injection was made, this time of 0.2 mL of 14%
perfluoropropane. Day-1 following the second injection found complete resolution of corneal oedema with closure of the intrastromal cleft. The vision had also improved and was consistent with the final visual acuity 8-weeks later.

Basu et al.\textsuperscript{26} in a retrospective study of acute corneal hydrops in keratoconus, PMCD and keratoglobus, compared 62 eyes treated with 14\% non-expansile, intracameral C\textsubscript{3}F\textsubscript{8} with 90 eyes that served as controls. There was significantly faster resolution of clinical oedema in the keratoconus group treated with intracameral C\textsubscript{3}F\textsubscript{8} than controls (67.6±39.2 versus 110.6±51.6 days; \(p<0.0001\)). However, 10 of the study eyes (16\%) developed acute glaucoma secondary to pupillary block compared to none of the control eyes (\(p<0.0001\)). This incidence of pupil block glaucoma prompted the investigators to perform surgical iridectomy in subsequent eyes treated with intracameral C\textsubscript{3}F\textsubscript{8}. In a subgroup of 21 study eyes and 19 control eyes no statistical difference was identified in terms of endothelial cell density, polymorphism or polymegathism following resolution of oedema. Similar to other studies, no difference in the final best-corrected visual acuity was identified between the two groups.

In a separate, smaller, study, Basu et al.\textsuperscript{8} assessed eyes with hydrops before and after intracameral C\textsubscript{3}F\textsubscript{8} with AS-OCT and noted that C\textsubscript{3}F\textsubscript{8} made little difference in the speed of resolution of hydrops in three out of 13 eyes treated with intracameral perfluoropropane. The average diameter of the Descemet’s tear in these three eyes was 2.1 ± 0.4 mm and the depth was 1.7 ± 0.1 mm; whereas, the average diameter of tear in all 24 hydrops corneas included in the study was 1.1 ± 0.8 mm with a mean depth of 0.9 ± 0.6 mm. The authors postulated that in eyes with large
and deep tears, intracameral gas injection may in fact impede re-attachment of Descemet’s membrane and thus slow recovery.

Rajaraman et al. proposed the use of compression corneal sutures in addition to intracameral perfluoropropane in the treatment of acute hydrops in a retrospective case series of seventeen patients (16 keratoconus and one PMCD). Patients either received 0.2 mL of iso-expansile mixture of 14% $C_3F_8$ alone, or with compression sutures. The decision to introduce compression sutures was only made after injection of the gas if a) a stromal cleft was noted after gas tamponade, or b) tracking of gas through the stroma was noted during gas injection. Fifteen of the patients underwent intracameral $C_3F_8$ with sutures and only two eyes underwent intracameral gas injection alone. The average persistence of the bubble in the anterior chamber was $10.75 \pm 2.62$ days. Corneal oedema resolved faster in eyes with $C_3F_8$ and compression sutures than eyes with pneumopexy alone ($8.87 \pm 4.98$ days and 27.5 days, respectively), however, the numbers are too small to be conclusive.

Histopathology of resolved hydrops in eight eyes treated with intracameral $C_3F_8$ injection showed greater attachment of Descemet’s membrane to the posterior stroma and “burial” of the rolled or folded Descemet’s membrane in the stroma. This appearance may reflect compression effects of the gas bubble on Descemet’s membrane. Separation between the split ends of Descemet’s membrane suggests that end-to-end re-approximation of Descemet’s membrane may not be possible because of the elastic coiling and retraction of Descemet’s
membrane after rupture. There were no signs of endothelial attenuation in eyes that received C$_3$F$_8$ on histology.$^8$

Whilst adverse events occur uncommonly in the above series, many anterior segment experts still advocate utilizing iso-expansile gases with caution, largely due to associated serious complications such as pupil block glaucoma,$^{26,30}$ intrastromal migration of gas,$^{31}$ Urrets-Zavalia syndrome,$^{32}$ and potential complications such as cataract and endothelial cell loss. Additionally, not every patient can adhere to the posturing regimen required as part of this treatment. Indeed, every study on intracameral gas injection for acute hydrops has prescribed supine positioning post-injection for a significant period up to two weeks. This could pose potential compliance issues for many patients. The use of intracameral gases also dictates more frequent follow-up as it is important to ensure complications such as malignant glaucoma and intrastromal migration of the gas do not occur. If a gas other than perfluoropropane is to be used then close observation is recommended, as repeated injections are frequently necessary.$^{23,24}$

The definitive benefit gained from intra-cameral gas across studies is approximately a one month faster resolution of hydrops, but no significant difference in terms of final best-corrected visual acuity or need for corneal transplantation.$^{23,26}$ Therefore, it may be advisable to first measure the dimensions of the Descemet’s tear with AS-OCT, and if the tear is of appropriate dimensions$^8$ then the procedure might be recommended for individuals who are highly compliant and motivated for faster resolution from hydrops and possibly earlier corneal transplantation. The limited evidence, suggests that intracameral
perfluoropropane may be the gas of choice as it requires the least number of re-injections and it has also been demonstrated by both in vivo and ex vivo studies to be safe in context of endothelial preservation.\textsuperscript{8,26} Further studies are required to validate the area and depth of the tear beyond which intracameral gas injection is unhelpful. From a personal perspective, due to issues of patient compliance, pupil block glaucoma, reinjection of gas and lack of benefit in terms of final visual acuity or reduction in need for transplantation surgery, the author has abandoned intracameral gas injection.

Traditionally penetrating keratoplasty has been employed for patients following acute hydrops as the resultant stromal scar was thought to preclude successful lamellar keratoplasty. Keratoconus has been shown to have one of the best outcomes for PKP.\textsuperscript{33} There are varied reports as to the success of PKP following acute hydrops. Akova et al. reported no significant difference in the rate of endothelial graft rejection in 35 eyes with a history of hydrops compared to 74 eyes that have not, despite a higher rate of vernal keratoconjunctivitis in the hydrops group.\textsuperscript{34} However, Tuft et al., in a larger study with a longer period of follow-up, reported that the success of PKP reduces in patients post-hydrops due to presence of neovascularisation and the higher prevalence of vernal conjunctivitis.\textsuperscript{13} Basu et al. conducted a similar study more recently that generally supported the study by Tuft et al., but using multivariate analysis identified that the risk of endothelial rejection episodes was greater in eyes with longer duration of corneal hydrops and coexistent ocular allergy.\textsuperscript{20}

Because of the greater risk of endothelial graft rejection and reduced success of
long-term graft survival noted in the preceding studies, and the typically young age of individuals who develop hydrops, recent years have seen the adoption of deep anterior lamellar keratoplasty (DALK) techniques wherever feasible for post-hydrops keratoconus patients. DALK poses technical challenges over those posed by PKP, largely due to the depth and density of scarring in severe cases and the significant risk of deep perforation at the site of the Descemet’s membrane rupture. Therefore many of the techniques described for lamellar dissection are contraindicated. Modified dissection techniques have been described in all published case series of DALK following hydrops, most suggesting careful manual dissection down to near-Descemet’s membrane.\textsuperscript{35,36}

In the largest, retrospective, non-comparative series of 22 post-hydrops keratoconic eyes treated with DALK published by Anwar et al.,\textsuperscript{35} in which a modified “big bubble” technique was utilized, the three-year postoperative follow up saw 68.1% of all eyes with a BSCVA of 20/40 or better, 27.2% had 20/30 or better, and 9.1% had 20/28 or better. No eyes achieved 20/20. The mean BSCVA at 3 years of follow-up was 20/40. The mean spherical equivalent was -3.53D, and the average refractive cylinder was 3.42D. Six eyes (27%) developed micro-perforations intraoperatively but none required conversion to PKP.

In a more recent conducted by Nanavaty et al.,\textsuperscript{36} ten keratoconic eyes of ten patients underwent DALK post hydrops. Preoperatively BSCVA was 20/80 or worse in all eyes. At one-year, 100% had BSCVA of 20/40 or better. Intraoperative micro-perforation at the site of previous hydrops occurred in six eyes (60%) but none required conversion to penetrating keratoplasty. At latest follow-up, mean
spherical equivalent was \(-2.4\) D and refractive astigmatism was 3.42 D. Mean central pachymetry was 572.6 µm. These excellent postoperative values are therefore comparable to results yielded by PKP post-hydrops, and with the benefit of longer rejection-free graft survival, DALK may soon become the “standard of care” for post-hydrops visual rehabilitation.

Our current approach to corneal hydrops, which is common and severe in our practice, is to treat with topical lubricants and corticosteroids (the latter to minimize inflammation and neovascularization not to address oedema) until resolution of the hydrops. Topical antibiotics are utilized where there is epithelial defect or the risk of such is high. We no longer use intracameral gas injection. At the time of corneal transplant our preference is to attempt DALK using a mechanical dissection technique in all cases where the Descemet’s tear is small (less than 3mm). Typically in larger tears a penetrating keratoplasty is performed.

### 4.8 Conclusions

Although relatively uncommon, acute hydrops in keratoconus is associated with significant morbidity in an otherwise healthy young population. Improved *in vivo* imaging of the cornea during acute hydrops has led to an enhanced understanding of the pathogenesis and ultrastructural changes of the condition. Anterior segment UBM and OCT in particular have resulted in improved management of the disease. However, despite newer treatment modalities which may shorten the duration of hydrops, ultimately the majority of affected subjects require corneal transplantation for visual rehabilitation.
Effective management of acute corneal hydrops in keratoconus is based on recognising and addressing the risk factors, treating the acute event effectively and promptly to reduce the duration of oedema and its complications, and ultimately successful corneal transplantation with acceptable long-term graft survival rates. Unfortunately, as highlighted in this perspective, the evidence base still lags behind surgeons’ enthusiasm for a number of management options and larger prospective studies are most definitely required to further refine possible prevention and treatment options for this multifaceted disease process.
Chapter 5

*Predictors of acute corneal hydrops in keratoconus: the Auckland keratoconus study*
5.1 Abstract

Aims
To identify potential factors associated with acute corneal hydrops in a New Zealand population with keratoconus referred to a hospital eye service.

Method
A single hospital centre, retrospective review, of demographic and clinical features of subjects with keratoconus and corneal hydrops, over a 17-year period, compared with an age- and gender- matched control group of subjects with keratoconus but no history of corneal hydrops.

Results
101 eyes of 101 subjects (mean age 24.6 ± 8.4 years) with keratoconus related corneal hydrops were identified. Subjects were more likely to be of Pacific, but less likely to be of New Zealand European, ethnicity than control subjects (N=101). In comparison, Maori ethnicity was not found to have a significantly positive or negative association with hydrops. The pre-hydrops best-corrected visual acuity (BCVA) of affected eyes was poorer than that of controls (p<0.001) at first presentation to our tertiary referral corneal and contact lens service. Hydrops typically developed approximately 4 years after diagnosis of keratoconus. Subjects with hydrops were more likely to have a history of eye-rubbing (p=0.011), but less likely to have a family history of keratoconus (p=0.05). In 31 cases the acute hydrops event was their first optometric/ophthalmology contact. There were
no statistically significant differences in the prevalence of atopic disease, contact lens wear, or overall corneal transplantation rate between the two groups.

**Conclusions**

Pacific ethnicity, history of eye-rubbing, poor BCVA at first hospital presentation and lack of family history were statistically associated with developing acute corneal hydrops in keratoconus in a New Zealand population. Greater understanding of such predisposing risk factors may help develop early management strategies to delay or prevent progression of this disease.
5.2 Introduction

Acute corneal hydrops is an incompletely understood complication of keratoconus, occurring in approximately 3% of keratoconus patients.\textsuperscript{13} It may also occur in other corneal ectasias, such as pellucid marginal degeneration and keratoglobus.\textsuperscript{37,38} Corneal hydrops develops following acute rupture of Descemet's membrane and overlying endothelium, that allows aqueous to enter the corneal stroma and epithelium, resulting in severe corneal oedema. In recent years, modern technology, such as \textit{in vivo} confocal microscopy (IVCM) and anterior segment optical coherence tomography (AS-OCT), has been able to elegantly demonstrate these features of acute corneal hydrops \textit{in vivo}.\textsuperscript{8,21}

Although acute corneal hydrops is usually self-limiting and clinical signs of oedema typically resolve after 2 - 4 months,\textsuperscript{9} it often leaves a vision-impairing scar, necessitating or expediting the need for corneal transplantation. Severe complications of corneal hydrops are infrequent but include: extensive corneal scarring, severe neovascularisation, epithelial defects, microbial keratitis, corneal perforation and glaucoma.\textsuperscript{11,39,40} A history of hydrops may also predispose subjects to greater likelihood of episodes of endothelial graft rejection after penetrating keratoplasty.\textsuperscript{20}

Keratoconus is the most common indication for corneal transplantation in Australasia, accounting for approximately half of the corneal transplants in New Zealand\textsuperscript{16} and almost a third of those performed in Australia.\textsuperscript{41} It is generally considered a bilateral disease\textsuperscript{42} that is suspected to be more prevalent in Maori
and Pacific peoples, in whom the condition also appears to be more aggressive.\textsuperscript{16} Whilst several risk factors for the development of hydrops have been postulated, including male gender,\textsuperscript{13} advanced corneal ectasia,\textsuperscript{13} co-existing vernal keratoconjunctivitis,\textsuperscript{9} and eccentric (rather than central) cone location,\textsuperscript{43} few studies have concentrated on the identification of potential predictors of the phenomenon.

To further understand the development of acute hydrops in the keratoconus population, and to identify potential risk factors we analysed the records of all patients diagnosed with acute corneal Hydrops, associated with keratoconus, who attended a hospital-based practice over a 17-year period. A particular focus was the demographic background and clinical characteristics of the affected subjects.

### 5.3 Methods

**Subject Recruitment and Assessment**

The records of all patients with keratoconus diagnosed with acute corneal hydrops who presented to the Cornea and Anterior Segment Service or the Contact Lens Service at the Department of Ophthalmology, Auckland City Hospital, during the period January 1 1990 – December 31 2007 (inclusive), were reviewed. Cases were identified through a computerised search for clinical diagnosis of “keratoconus” and/or “other corneal hydrops” under the hospital’s electronic clinical coding system. The clinical diagnosis of acute corneal hydrops was defined as the sudden onset of bullous corneal oedema in the context of a subject with keratoconus. The diagnostic criteria of keratoconus were consistent with that
of the Collaborative Longitudinal Evaluation of Keratoconus (CLEK) study. Where both eyes had been affected by hydrops, only the first affected eye was included in the analysis. The study adhered to the tenets of the declaration of Helsinki.

For all subjects, demographic information (age, gender, and ethnicity), ocular history, in particular whether there was a history of contact lens use in the year leading up to the development of hydrops, history of atopy (defined as at least two of: asthma, atopic dermatitis or allergic rhino-conjunctivitis), and family history of keratoconus in a first- or second-degree relative were recorded. It is not uncommon for people to belong to more than one ethnic group, however, upon first presentation to public hospital services in New Zealand, patients are asked to report their primary ethnicity. This is recorded for all subjects and was used for ethnicity related analyses in this study. The best-corrected Snellen visual acuity was recorded for the first hospital visit for both eyes but converted to logMAR for ease of statistical analysis. The date of onset of acute hydrops, clinical history of the onset of hydrops including history of eye-rubbing (defined simply as “no”, “frequently” or “a lot”), complications, management and outcome were also recorded. Where information regarding ocular history or patient demographics was missing, following verbal consent, subjects were interviewed by telephone to retrieve the relevant facts.

Subjects diagnosed with acute corneal hydrops due to other corneal ectasia or following penetrating keratoplasty were excluded. Subjects whose records were
incomplete and could not be completed by telephone interview were also excluded.

A group of subjects with keratoconus, but no history of acute Hydrops, was selected on the basis of similar age (at first presentation to the hospital eye department for tertiary assessment) and gender spread. The control group was identified by computerised search of the hospital clinical coding database and the Contact Lens Clinic database using the sole search term “keratoconus” (Department of Ophthalmology, Auckland City Hospital). Only one eye per subject (selected at random) was included for analysis. Identical information to that obtained for the study group was obtained for the control group from clinical records, and again, where any information was missing, subjects were interviewed by telephone to retrieve the relevant facts. By study design, this was a selective keratoconus group who had been referred to the hospital service for contact lens provision or anterior segment specialist assessment.

Statistical Analysis
All values were entered into a database (Excel; Microsoft, Redmond, WA) and subsequently imported into statistical software for analysis (SPSS, ver. 15 for Windows; Chicago, IL) with the assistance of a professional biomedical statistician. Basic descriptive statistics were calculated on all data gathered and are reported as mean ± standard deviation or n (%), as appropriate. Binary logistic regression multivariate analysis was performed to investigate factors influencing the development of acute corneal hydrops in keratoconus. Explanatory variables included were age, gender, ethnicity (self-defined), age at diagnosis of
keratoconus, earliest recorded visual acuity, history of atopy, history of eye-rubbing, past contact lens wear, and family history of keratoconus.

All tests were two-tailed and p-values are quoted where appropriate.

5.4 Results

In total 101 eyes of 101 subjects with a history of hydrops who fulfilled all study criteria were included for analysis. Fifteen additional patients were excluded from further study due to incomplete data or diagnosis of hydrops secondary to other ectasia and the remainder of the patient records did not contain adequate information to satisfactorily fulfil a diagnosis of acute onset corneal hydrops (in total approximately 800 patient records were reviewed for inclusion/exclusion purposes).

Table 5.1 Demographic and clinical factors for both corneal hydrops study group and the hydrops-free keratoconus control group (next page).
<table>
<thead>
<tr>
<th>Clinical factors</th>
<th>Hydrops</th>
<th>Control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (%)</td>
<td></td>
<td></td>
<td>0.754</td>
</tr>
<tr>
<td>- Male</td>
<td>55</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>- Female</td>
<td>45</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
<td></td>
<td>0.082</td>
</tr>
<tr>
<td>- New Zealand European</td>
<td>13</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>- Maori</td>
<td>25</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>- Pacific Island</td>
<td>54</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>- Other</td>
<td>8</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis of keratoconus (years)</td>
<td>20.3 ± 7.8</td>
<td>21.9 ± 7.1</td>
<td>0.943</td>
</tr>
<tr>
<td>Eye-rubbing (%)</td>
<td>75</td>
<td>55</td>
<td>0.011</td>
</tr>
<tr>
<td>Contact lens wear (%)</td>
<td>56</td>
<td>70</td>
<td>0.139</td>
</tr>
<tr>
<td>Atopy (%)</td>
<td>59</td>
<td>55</td>
<td>0.898</td>
</tr>
<tr>
<td>Family history of keratoconus (%)</td>
<td>24</td>
<td>43</td>
<td>0.050</td>
</tr>
<tr>
<td>Mean initial hospital-recorded BCVA of affected eye (LogMAR)</td>
<td>1.14 ± 0.63</td>
<td>0.63 ± 0.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean initial hospital-recorded BCVA of contralateral eye (LogMAR)</td>
<td>0.45 ± 0.41</td>
<td>0.31 ± 0.31</td>
<td>0.125</td>
</tr>
<tr>
<td>Penetrating keratoplasty of affected eye (%)</td>
<td>67</td>
<td>61</td>
<td>0.865</td>
</tr>
<tr>
<td>Penetrating keratoplasty of contralateral eye (%)</td>
<td>30</td>
<td>27</td>
<td>0.683</td>
</tr>
<tr>
<td>Ethnicities of subjects having undergone penetrating keratoplasty in one or both eyes (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- New Zealand European</td>
<td>10</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>- Maori</td>
<td>18</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>- Pacific Island</td>
<td>43</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>- Other</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

BCVA = best-corrected visual acuity
At the time of study the mean age of the 101 subjects that met the study criteria was 29.7 ± 9.1 years. The male:female ratio was 55:45. An identical number of patients (N=101) with a history of keratoconus, but without corneal hydrops, constituted the control group. This group was not statistically different in terms of age and gender match compared to the hydrops group (p=0.535 and p=0.754, respectively).

The mean age at diagnosis of keratoconus in the hydrops group was 20.3 ± 7.8 years (range 10 - 47 years); hydrops typically developed 4 years after diagnosis (mean age 24.6 ± 8.4 years). The mean age at diagnosis of keratoconus did not differ significantly between the hydrops and control group (21.9 ± 7.1 years) (p=0.943). Thirty-one of the 101 subjects in the hydrops group made their first presentation to eye care services with the episode of acute corneal hydrops; 80% of these subjects were of either Maori or Pacific Island ethnicity.
In the corneal hydrops group, 54% of subjects were of Pacific ethnicity, 25% Maori, 13% New Zealand European, and 8% of other ethnicity. Compared to the control subjects, the association of ethnicity with hydrops approaches significance (p=0.082). When analysed individually, associations reside particularly in those of Pacific Island or New Zealand European descent – with significantly more Pacific peoples (p=0.026) and less New Zealand Europeans (p=0.013) in the hydrops group compared to the control group. The number of Maori in each group did not differ statistically (p=0.134).

Eye-rubbing was significantly more common in the hydrops group (75%) compared to controls (53%) (p=0.011). Fifty-six percent had worn contact lenses prior to the onset of hydrops compared to 70% of control subjects (p=0.139). There was no significant difference in the history of atopy between the groups (p=0.577). The significance of these three variables is independent of each other. However, when comparing family history, those with a history of hydrops were less likely to have family members with keratoconus (24%) compared to those in the control group (43%) (p=0.05). Additionally, and perhaps unsurprisingly, those who presented with hydrops without a pre-existing diagnosis of keratoconus were much less likely to have worn contact lenses (37%) compared to patients who presented with hydrops with a pre-existing diagnosis of keratoconus (65%) or control subjects (70%) (p=0.05). No other differences in clinical features were identified between those who presented with hydrops compared to those who developed hydrops with known keratoconus.
Figure 5.2  Acute corneal hydrops in a 17 year old subject (a) with severe corneal and stromal oedema highlighted by (b) laser in vivo confocal microscopy (IVCM). Lower images highlight resolving acute corneal Hydrops with extensive central corneal scarring and significant corneal stromal neovascularisation (c) with IVCM revealing well-formed stromal blood vessels (d) containing red blood cells and highly reflective white blood cells.

The mean initial logMAR best-corrected visual acuity (BCVA) recorded for eyes subsequently affected by hydrops was 1.14 ± 0.63 (Snellen 20/280); this was significantly worse than the mean earliest logMAR BCVA recorded in the control group (0.63 ± 0.46) (Snellen 20/80) (p<0.001). Excluding the 31 subjects who
presented to the eye care services for the first time with acute hydrops, those in the hydrops group still had significantly worse initial logMAR BCVA compared to the control group (1.09 ± 0.63) (Snellen 20/250) (p<0.001). The mean earliest recorded logMAR BCVA of the contralateral eye for subjects in the hydrops group was 0.45 ± 0.41 (Snellen 20/60); this did not differ significantly from the contralateral eye of subjects in the control group (0.31 ± 0.31) (Snellen 20/40) (p=0.125).

At the time of the study, 67% of the eyes with acute hydrops had undergone penetrating keratoplasty; this was not significantly different from the control group (61%) (p=0.865). Thirty percent of contralateral eyes in the hydrops group had also undergone penetrating keratoplasty compared to 27% of the contralateral eyes in the control group (p=0.683). Of those subjects with a history of hydrops requiring penetrating keratoplasty in one or both eyes (n=79), 43 were of Pacific ethnicity, 18 Maori, 10 New Zealand Europeans, and 10 were of other ethnicities. In the control group, 69 subjects required penetrating keratoplasty in one or both eyes, of whom 21 were of Pacific ethnicity, 15 Maori, 25 New Zealand Europeans, and 8 of other ethnic groups. Therefore, of all subjects requiring penetrating keratoplasty in this study, 65% were either Pacific peoples or Maori, and 24% New Zealand Europeans.

These variables and key statistics are summarised in table 5.1. Illustrative clinical features of subjects from the acute hydrops group are shown in figures 5.1-4.
Figure 5.3  Resolved corneal hydrops associated with keratoconus in two subjects: (a) a relatively small, off axis, corneal scar associated with significant irregular astigmatism and poor best contact lens corrected vision; and (b) an extensive central and inferior, anterior stromal, corneal scar reducing best corrected vision to 6/36 (early cataract is also observed in this image).

(Images courtesy of Professor McGhee)

5.5 Discussion

Keratoconus leading to penetrating keratoplasty appears to be of higher prevalence in New Zealand than in other developed nations\textsuperscript{16} and on the basis of clinical experience, but not yet published data, the severity of keratoconus is also thought to be greater in New Zealand. This component of the Auckland Keratoconus Study aimed to identify demographic and clinical risk factors in the New Zealand population that may predispose to acute corneal hydrops in keratoconus, by comparing a set of variables in subjects with a history of acute
corneal hydrops with an age and gender matched control group of keratoconus subjects without hydrops.

In the study population of 101 subjects with acute corneal hydrops, a trend between ethnicity and acute hydrops was identified, with Pacific peoples more likely, and New Zealand Europeans less likely, to develop acute hydrops. A history of eye-rubbing, poor BCVA at first hospital presentation and, paradoxically, a negative family history of keratoconus, were also associated with acute hydrops.

While atopy or atopic syndrome is typically defined as “A familial tendency to produce IgE antibodies in response to low doses of allergens and to develop typical symptoms, such as asthma, rhinoconjunctivitis or eczema/dermatitis,” the majority of the subjects encountered in this study had not been serologically assessed for diagnosis of atopic disease. Therefore, to avoid over-inclusion, patients in this study required a history of two of the three common clinical manifestations to be assigned as atopic. An association, but not causality, between corneal hydrops and atopic keratoconjunctivitis has been noted, yet a history of atopy in the current population did not appear to be associated with hydrops. One possible explanation for this, despite the size of the study groups, is that atopy per se is common in the New Zealand population and particularly in the keratoconic population. Notably, although no statistically significant predisposition could be elucidated in this study, atopic disease was highly prevalent in both groups.
Figure 5.4  Histology of a corneal button obtained from a subject undergoing penetrating keratoplasty for keratoconus associated with prior acute corneal hydrops. Although oedema has resolved the retracted Descemet’s membrane is clearly visible (large arrow head) and the area of Descemet’s absence in the initial tear is still visible (small arrow) with decreased keratocyte density in the overlying posterior stroma. The epithelium shows variable thickness with areas of Bowman’s layer loss and fibrotic scar extending into the epithelium (small arrowhead). (Congo red stain, original magnification x40). (Image courtesy of Trevor Sherwin)

We identified a statistically significant association between eye rubbing and acute hydrops. A history of eye-rubbing has previously been revealed to be significantly associated with hydrops, and to be an independent risk factor from contact lens wear and atopy – both potentially confounding variables that in the past have been linked to eye-rubbing.\textsuperscript{49-51} However, in the current study we recognise that there
could be potential recall bias following an event such as acute hydrops and that
the classification of severity of eye rubbing may not have been consistent in
clinical records, both potential limitations in terms of subsequent analysis.

The current study demonstrated that hydrops is strongly associated with poor
BCVA at first hospital presentation compared to control subjects, even when the
31% of subjects who first presented to hospital eye services with acute hydrops
were removed from analysis. Unfortunately, these data confirm the clinical
impression that it is not uncommon in New Zealand for the diagnosis of
keratoconus to be made late, when patients present with advanced keratoconus
or hydrops despite having had poor visual acuity for several years. Despite a “free
at point of access” public health service (similar to the British National Health
Service (NHS)) the exact number of such cases and reasons for such late
presentation for treatment has yet to be established.

The genetic predisposition to the development of keratoconus is well-established
despite being incompletely understood.\textsuperscript{12} Not only is there a familial association in
keratoconus, certain ethnic groups have been found to have a higher prevalence
of the disease than others within the same geographical region.\textsuperscript{52-54} Interestingly,
the current study suggests that those who develop hydrops are more likely to be
of Pacific ethnicity, and less likely to be of European descent, compared to the
control keratoconic population. A previous study has shown that the proportions of
European, Pacific and Maori patients attending the tertiary corneal clinic are
comparable to that of the New Zealand population.\textsuperscript{55} However, patients diagnosed
with keratoconus had significantly higher proportions of Maori and Pacific patients
and lower rates of European patients than the total population. This suggests that
disease specific factors play a role in the ethnic distribution of patients with
kera... Pacific peoples making up only around 7% of the New Zealand population, of corneal transplants performed in this study were on Pacific subjects (with or without a history of hydrops). The authors believe this to be representative of the overall New Zealand population as approximately half of all corneal transplantation procedures in New Zealand are performed in the Auckland metropolitan area. Furthermore, the public health service, represented by Auckland District Health Board Ophthalmology Department in this study, performs the majority (71.8%) of corneal transplants in the Auckland region. Somewhat counter-intuitively, in the current study corneal hydrops was statistically less common in those with a family history of keratoconus in a first or second degree relative. This observation is also supported by the New Zealand data published by Jordan et al. (2011) that highlighted a significant difference in the tomographic characteristics of subjects with family history of keratoconus and those without. However, in the context of the current study it must be noted: firstly, the assessment of family history was based on self-reporting, secondly, although the prevalence of family history was much higher in both groups than in many published studies the majority of subjects did not report a family history, and finally, it is conceivable that a family history of keratoconus may tend to “protect” against more severe keratoconus and hydrops in terms of (evolutionary) survival.
Perhaps somewhat paradoxically, subjects with hydrops were no more likely to have had a history of contact lens wear than those without hydrops. This observation is unusual, since the advanced keratoconus that predisposes to corneal hydrops generally leads to greater reliance on rigid contact lens wear for visual rehabilitation and is also inconsistent with the hypothesis that contact lens wear itself is potentially a cause of mechanical trauma and keratocyte apoptosis that may contribute to disease progression and development of hydrops.\(^4,12,61\) A possible explanation for this difference is that a) in almost one third of the cases this was the first presentation to hospital eye services (the main source of subsidised contact lens provision for keratoconus in New Zealand) and b) the hydrops subjects had more advanced ectatic disease in the year prior to the acute event compared to controls and may therefore have been more likely to be intolerant of contact lens wear.

Since the control study group were referred to hospital services with advanced keratoconus, perhaps unsurprisingly, the hydrops subjects were not more likely to undergo penetrating keratoplasty in the affected eye compared to hydrops-free subjects. In contrast, Tuft et al. reported the development of acute hydrops to be strongly associated with having a subsequent penetrating keratoplasty (59.2% of hydrops subjects versus 13.1% of hydrops-free subjects, \(p=0.00001\)).\(^13\) The reason for the differences in this study may lie in the fact that the non- hydrops subjects with keratoconus were recruited from the hospital optometry and anterior segment clinics. Therefore these are typically patients with advanced keratoconus referred from the community for specialist optometric care or consideration of a corneal transplant.
In conclusion, in the current study, acute corneal hydrops in keratoconus was statistically associated with Pacific but not Maori ethnicity and appeared less frequently in those of New Zealand European ethnicity. Hydrops also appeared to be associated with a history of eye-rubbing but not with a family history of keratoconus. Those who progressed to hydrops tended to have significantly worse visual acuity at first hospital presentation; however, late presentation to hospital services with advanced keratoconus was not uncommon. Corneal transplantation did not appear more common in the hydrops population compared to a matched non-hydrops hospital population with advanced keratoconus. Although we believe this large retrospective study involved a representative sample with assessment of 202 New Zealand subjects with advanced keratoconus, we envisage that a forthcoming, larger, prospective, clinical study of the natural history of subjects with keratoconus in New Zealand may further elucidate key associations.
Chapter 6

*A prospective study of acute corneal hydrops by in vivo confocal microscopy in a New Zealand population with keratoconus*
6.1 Abstract

Purpose
To analyse the clinical and microstructural changes during the course of acute corneal hydrops in keratoconus.

Methods
A prospective study of consecutive patients presenting with acute corneal hydrops over 12 months was performed. Patients were examined with slit-lamp biomicroscopy and in vivo confocal microscopy (IVCM) at two-weekly intervals until resolution of hydrops.

Results
Ten consecutive patients (mean age 23.6±7.5 years) were recruited. All were of Maori or Pacific Island Nations origin. All affected eyes exhibited extensive grade 3 acute corneal hydrops. Mean best-corrected vision in hydrops eyes was 2/60 at presentation and 6/48 at resolution. Mean time to resolution of hydrops was 9.9±4.3 weeks.

On IVCM, four corneas exhibited hyper-reflective round cells in the epithelium and stroma. Elongated branching cells with small cell bodies were noted in the anterior stroma in 2 cases at 6 and 12 weeks respectively. Three months after presentation, both cases also exhibited unusual stromal cells with large speckled cell bodies and elongated branching cell processes. Both cases subsequently developed corneal neovascularisation.
Conclusions

Corneal neovascularisation occurred in 20% of eyes in this pilot study and prolonged presence of presumed inflammatory cells was observed in these eyes. Further study of this potential association may enable identification of patients at risk of neovascularisation.
6.2 Introduction

Keratoconus is an ectatic disorder of the cornea, classically described as progressive, non-inflammatory and characterised by central corneal thinning, corneal protrusion, and irregular myopic astigmatism.\(^{62}\)

Acute corneal hydrops (the development of marked corneal oedema due to a break in Descemet’s membrane) occurs in 2.4 – 3.0% of eyes with keratoconus, typically causing a sudden further deterioration in vision.\(^{13,26,63}\)

The risk factors for developing acute corneal hydrops mirror those for keratoconus. These include eye rubbing, atopy and early age of onset.\(^{51,64,65}\)

Complications of acute corneal hydrops include corneal neovascularisation, intrastromal clefts, infection and even corneal perforation.\(^{13,63}\)

Unfortunately, there are only a limited number of clinical studies specifically investigating acute corneal hydrops in keratoconus, and these tend to be retrospective.\(^{13,26,63}\) Therefore, the aims of this study were to prospectively investigate the clinical course of acute corneal hydrops and to use in vivo confocal microscopy (IVCM) to observe micro-structural corneal changes over time in these eyes.

6.3 Materials and methods

Subjects
A prospective, observational, clinical study of consecutive cases of acute corneal hydrops in keratoconus, presenting over a one year period, was conducted. All patients were recruited from initial presentation to the emergency eye services at the Department of Ophthalmology, Greenlane Clinical Centre, Auckland District Health Board, Auckland, New Zealand and managed by the University of Auckland Cornea and Anterior Segment unit.

Inclusion criteria included: presentation within 48 hours of onset of symptoms of acute corneal hydrops, and either a pre-existing history of keratoconus or clinical signs of keratoconus in the fellow eye. Exclusion criteria included corneal hydrops due to other corneal ectasias, a previous history of ocular trauma or previous ocular surgery.

Informed consent was obtained from all participants and the study design adhered to the tenets of the Declaration of Helsinki. The study protocol was approved by the Northern X regional ethics committee.

**Methods/Assessments**
After initial enrolment and assessment, all participants were examined at 2 weekly intervals until resolution of the acute hydrops (defined as absence of corneal oedema on slit-lamp biomicroscopy).

Visual acuity was assessed using a Snellen chart (converted to logMar for purposes of statistical analysis). Slit lamp biomicroscopy was performed on all eyes and in all cases Orbscan II tomography (Bausch and Lomb Surgical,
Rochester, NY, USA) was performed to confirm the clinical diagnosis of keratoconus in the contralateral eye. In those cases with a corneal transplant in the fellow eye, the clinical case notes were reviewed to confirm the diagnosis. In all cases where prior clinical notes were available, before presentation with acute hydrops, these were assessed to confirm the history of keratoconus.

The severity of corneal hydrops was graded according to the extent of corneal oedema; grade 1 within a circle of 3 mm diameter, grade 2 between circles of 3 and 5 mm diameters, and grade 3 larger than a circle of 5 mm diameter.\textsuperscript{13}

Laser scanning \textit{in vivo} confocal microscopy was subsequently performed on all subjects using the Heidelberg Retina Tomograph II Rostock Corneal Module (RCM) (Heidelberg Engineering GmbH, Germany). A 60x objective water immersion lens with a numerical aperture of 0.9 (Olympus, Japan) and a working distance, relative to the applanating cap, of 0.0 to 3.0 mm was used. All eyes were anesthetised using a drop of 0.4% benoxinate hydrochloride (Chauvin Pharmaceuticals, Kingston-upon-Thames, England). Viscotears (Carbomer 980, 0.2%, Novartis, Australia) was used as a coupling agent between the applanating lens cap and the cornea. During the examination, all subjects were asked to fixate on a distance target aligned to enable examination of the central cornea. The full thickness of the central cornea was scanned using the device’s “section mode”. The section mode enables instantaneous imaging of a single area of the cornea at a desired depth. Images at varying depths were obtained by manually adjusting the depth of focus. The overall examination took approximately 10 minutes to
perform for each subject and none of the subjects experienced any corneal complications as a result of examination.

All images were reviewed and analysed by an experienced examiner (DVP). Measurements were performed using a calliper tool (analySIS 3.1, Soft Imaging System, Münster, Germany).

6.4 Results

Clinical data

Twelve patients that met all inclusion criteria were identified and recruited over the twelve-month study period, however, two subjects failed to attend follow-up assessments following initial recruitment and were therefore excluded from analysis. Thus a total of ten patients (5 male, 5 female) were included and followed up within the 12-month study period. The mean age was 23.6 ± 7.5 years and all were of Maori or Pacific Island Nations origin (7 Samoan, 2 Maori, 1 Nuiean). All cases presented with unilateral acute corneal hydrops, although one patient (case j) had a previous history of hydrops in the contralateral eye resulting in corneal scarring and neovascularisation, and 4 others had a history of previous penetrating keratoplasty in the contralateral eye. The right eye was affected in 5 cases and the left eye affected in 5 cases. Six patients reported a history of atopy and 8 reported previous contact lens wear. Eight subjects reported frequent eye rubbing behaviour.
Figure 6.1  Clinical images of acute corneal hydrops in 10 eyes at initial presentation and at resolution. Cases g and j developed stromal neovascularisation.

All patients presented with extensive grade 3 acute corneal hydrops (area involved greater than 5mm diameter). The mean uncorrected visual acuity (UCVA) at presentation in the affected eye was $1.85 \pm 0.30$ logMAR (Snellen equivalent 1/60) and the mean best- corrected visual acuity (BCVA) was $1.68 \pm 0.48$ logMAR (Snellen equivalent 2/60).
Nine patients were treated with topical ocular corticosteroid drops at presentation. Five eyes were treated with potent corticosteroids; prednisolone acetate 1% (Pred Forte; Allergan, Irvine, California) or dexamethasone 0.1% (Maxidex; Alcon, Fort Worth, Texas), whereas, four eyes were treated with less potent glucocorticoid in the form of fluorometholone 0.1% (FML, Allergan; or Flucon, Alcon). The remaining eye was treated with topical ocular lubricants alone.

The mean follow-up period, which equated to mean time to resolution of hydrops, was 9.9 ± 4.3 weeks (range 4 – 18 weeks). The mean UCVA and BCVA at resolution of hydrops was 1.1 ± 0.48 logMAR (Snellen equivalent 6/60) and 0.91 ± 0.53 logMAR (Snellen equivalent 6/48) respectively.

Slit lamp photographs of all corneas at presentation and at resolution of hydrops are shown in Figure 6.1.

Nine eyes subsequently underwent penetrating keratoplasty for visual rehabilitation.

**Laser scanning in vivo confocal microscopy**

A total of 15,127 IVCM images were obtained, with a mean of 240 ± 105 images obtained per session.

All corneas exhibited intraepithelial bullae, observed as dark circular regions with well-defined margins. These cystic spaces ranged from 5µm to 300µm in diameter.
and were predominantly located at the level of the superficial epithelium and wing cell layers (Figure 6.2).

In 2 cases, epithelial bullae were still visible on IVCM at the final IVCM examination (28 days and 81 days) despite clinical resolution of oedema. For the remaining 8 cases, epithelial bullae had resolved on IVCM at a mean of 53 ± 25 days (range 14-90 days) after presentation.

In 6 cases stromal keratocytes could be clearly imaged and exhibited reflective cell bodies with well-defined cell borders. Intercellular hypo-reflective lacunae were present and appeared to have larger diameters in the mid stroma (approximately 10-100µm) compared to the anterior stroma (approximately 10-40µm) (Figure 6.3). Keratocyte nuclei were not clearly visible. In the remaining 4 cases, stromal keratocytes could not be clearly imaged due to the degree of corneal stromal oedema.

**Figure 6.2**  *In vivo* confocal microscopy images (figure on next page) showing corneal epithelial bullae in the superficial and wing cell layers (a-c). Bullae are absent at the level of the basal epithelium (d). Oblique sections (e,f) showing intraepithelial bullae predominantly located at the level of the superficial epithelium and wing cell layers.
All corneas exhibited hyper-reflective band like structures in the anterior stroma extending from approximately 50µm to 120µm depth (Figure 6.4). The presence of “islands” of basal epithelial cells between these bands suggested irregularity of the epithelial-stromal interface and this was confirmed in non-tangential images (Figure 6.4b).

All corneas exhibited microfolds in the anterior and mid stroma (Fig 6.5) and these were observed at presentation in 7 cases, and at 2 weeks, 6 weeks and 8 weeks in the remaining 3 cases respectively.

**Figure 6.3**  *In vivo* confocal microscopy images showing keratocytes with reflective cell bodies, well-defined cell borders, and intercellular lacunae in the anterior stroma (a) and mid stroma (b).

In 6 cases, (presumed) inflammatory cells were not visible at any time-point during the study (i.e. cells other than corneal epithelial cells, keratocytes or corneal endothelial cells). However, the remaining 4 cases exhibited hyper-reflective
round cells (5-10µm diameter) in the epithelium and anterior to mid stroma (Fig 6.6a, b). In two of these cases, the cells were only present for 2 to 4 weeks, but persisted in the remaining two cases (Fig 6.1, cases g and j). Elongated branching cells with small cell bodies (7-10µm diameter) were noted in the anterior stroma in 2 cases (g and j) at 6 and 12 weeks respectively (Fig 6.6c, d). Some of these cells appeared to be aligned along stromal bands (Fig 6.6d). Three months after presentation, both of these cases also exhibited unusual stromal cells with large, round, speckled cell bodies (15-30µm diameter) as well as elongated branching cells with small cell bodies (Fig 6.6e, f). In some areas, these two cell types appear to overlap, giving the appearance of a round cell with branching extensions. Both of these cases (g and j) developed corneal neovascularisation and were the only corneas to do so during the study. Interestingly, case g did not exhibit any inflammatory cells in the contralateral eye on IVCM at presentation. Case j had a previous history of hydrops in the contralateral eye resulting in corneal scarring and neovascularisation, and exhibited elongated branching cells with small cell bodies on IVCM.
Figure 6.4  *In vivo* confocal microscopy images showing hyper-reflective band-like structures in the anterior stroma with islands of basal epithelial cells (a,c,d). An oblique section confirms an irregular, hyper-reflective epithelial-stromal interface (b).
Figure 6.5  *In vivo* confocal microscopy images showing microfolds in the anterior and mid stroma

![Confocal microscopy images](image)

**Figure 6.6**  *In vivo* confocal microscopy images (figure on next page) showing hyper-reflective round cells (a,b), elongated branching cells (c,d), and cells with large speckled bodies and elongated branching processes (e,f). Images a,c and e were from case g, and images b,d and f were from case j.
Discussion

Corneal hydrops is typically associated with an acute, severe, reduction in visual acuity and function due to corneal oedema, and profound visual impairment was observed in all eyes in the current study (mean presenting BCVA in the affected eye was 20/800). It is now generally accepted that corneal stromal oedema due to hydrops is self-limiting, when migration of corneal endothelial cells adequately (re)cover the exposed stroma. This recovery process has been reported to take between 3 to 6 months. In the current study, the mean time to resolution of hydrops was 9.9 ± 4.3 weeks (range 4 – 18 weeks), with the corneal oedema fully resolved on clinical examination within 20 weeks in all cases.

Studies have suggested that between 31-63% of eyes with acute hydrops recover useful vision with contact lenses. However, in the current prospective study, only one eye achieved a BCVA approaching New Zealand driving standard (20/40) in the affected eye by the end of the study period.

Interestingly, all the subjects with acute corneal hydrops in the current study were of Maori or Pacific Island Nations origin, and these ethnicities have previously been shown to be over-represented in the keratoconic population in New Zealand. In this respect, two-thirds of the New Zealand population are of European origin and Maori and Pacific peoples make up approximately 20% of the population (Statistics New Zealand, Census 2006). However, in the Auckland Keratoconus study 65% of all subjects requiring penetrating keratoplasty during a 17- year period were either Maori or Pacific Island Nations origin (for comparison,
24% were New Zealand European).\textsuperscript{69} Keratoconus appears to be more aggressive in these two ethnic groups, and the registry data for indications for corneal transplantation in New Zealand support this observation.\textsuperscript{70}

It has also been previously observed that the Maori or Pacific Island Nations ethnic groups trend towards more severe asthma and atopy; conditions which may be associated with earlier onset of keratoconus.\textsuperscript{24} Our clinical experience in a tertiary centre with a large keratoconic patient base, is that these populations tend to develop severe grade 3 extensive hydrops (an area larger than a 5mm diameter circle) and have a greater propensity to corneal neovascularisation compared to European ethnic groups in New Zealand. These longstanding clinical impressions appear consistent with the data in the current prospective study. Variation in the severity of acute hydrops and subsequent corneal neovascularisation has also been reported in other ethnic groups, such as Saudi Arabian and Indian populations.\textsuperscript{51,71}

A variety of imaging techniques have been used to investigate acute corneal hydrops. An ultrasound biomicroscopy case series identified intrastromal clefts in all 13 eyes with acute corneal hydrops. The authors postulated that these clefts increase the surface area exposed to aqueous, resulting in the development of severe oedema.\textsuperscript{72} A recent Anterior Segment Optical Coherence Tomography (OCT) study in hydrops further supported the theory that resolution of hydrops requires reattachment of Descemet’s membrane and migration of corneal endothelial cells.\textsuperscript{8} This study highlighted that the duration of corneal oedema was
related to the dimensions of the Descemet’s membrane break and the depth (elevation) of the DM detachment from the stroma.

The literature regarding in vivo confocal microscopy of acute corneal hydrops currently only consists of single case reports. The current study is the first to prospectively follow acute corneal hydrops by performing serial IVCM examinations from presentation to resolution.

The epithelial bullae and stromal keratocyte appearances in this study are consistent with IVCM signs of corneal oedema described by Alomar et al. The irregular epithelial-stromal interface and presence of anterior stromal hyper-reflective bands in the current study also correspond with the sub-epithelial fibrosis observed in studies of corneas with chronic corneal oedema due to Fuchs’ endothelial dystrophy.

Corneal neovascularisation has significant implications on the patient’s future management and prognosis. In the current study, two patients developed corneal neovascularisation despite early treatment with topical corticosteroids. Both presented with corneal oedema extending close to the limbus, however, three other cases with similarly extensive oedema did not develop neovascularisation. Previous studies suggest that oedema near the limbus and intrastromal cleft formation in cases of acute corneal hydrops may be considered risk factors for subsequent stromal neovascularisation. Any associated inflammatory response, which may be greater in patients with atopy, has also been suggested as a potential stimulus to neovascularisation.
Interestingly, presumed inflammatory cells were not observed in the majority of cases at presentation or throughout the current study. Of the four eyes that did exhibit putative inflammatory cells, these were only present for 2 to 4 weeks in two of the cases. However, these cells persisted in the remaining two eyes, both of which went on to develop corneal neovascularisation. This may reflect the observations of Fromer and Klintworth who demonstrated that neovascularisation is usually preceded by leukocytic infiltration and that, conversely, the neovascular response is inhibited in animal models rendered leukopaenic by bone marrow irradiation.\textsuperscript{78,79}

Dendritiform cells (presumed Langerhans cells) have been reported to be present at the level of the epithelium and Bowman’s layer in approximately 30% of healthy individuals.\textsuperscript{80} Although these cells are not usually observed in the stroma in the healthy eye when imaged by \textit{in vivo} confocal microscopy, they may become apparent in the inflamed eye. The elongated branching cells with small cell bodies described in this study may therefore represent stromal Langerhans antigen presenting cells. The identity of the three cell types observed in the current study is uncertain and can only be postulated, but they may represent leukocytes, fibroblasts, vascular endothelial cells or pericytes. Together, these observations suggest that the presence of these cells on IVCM in acute hydrops is a potential risk factor for the development of corneal stromal neovascularisation. This may therefore provide a useful aid in targeting the management of these cases.
The use of topical corticosteroids may theoretically reduce the risk of corneal neovascularization or lessen the extent of progression should neovascularization occur. However, there is little evidence in the literature to support this theory. Indeed, although widely used in clinical practice, some studies have found topical corticosteroids to be entirely ineffective in arresting the progression of stromal neovascularization in corneal hydrops.\textsuperscript{22,77} Despite the ready access of corticosteroids into the corneal stroma, this limited efficacy has led to the suggestion of using systemic corticosteroids in high risk cases.\textsuperscript{77} As the clinical indications for use of anti-vascular endothelial growth factor (anti-VEGF) agents in corneal diseases increase, future studies may investigate their role in the potential management of intractable neovascularisation after acute corneal hydrops.\textsuperscript{81}

In conclusion, this study has demonstrated a mean time to resolution of hydrops of 11 weeks and also identified an over-representation of Maori and Pacific Island Nation ethnicities. Twenty percent of eyes in this pilot study developed corneal neovascularisation and IVCM confirmed the prolonged presence of presumed inflammatory cells in these eyes. If this association is confirmed by further study, IVCM may enable the identification of patients with acute corneal hydrops who are at risk of neovascularisation.
Chapter 7

An immunohistochemical study of acute hydrops in keratoconus
6.1 Abstract

Purpose
Keratoconus is traditionally considered a non-inflammatory condition, however, the corneal milieu is transformed by the onset of acute corneal hydrops. This study investigated cellular and microstructural changes in keratoconus-related acute corneal hydrops following clinical resolution of oedema, using immunohistochemical analysis.

Methods
This was an observational study of ten patients with acute corneal hydrops secondary to keratoconus. Trephined corneal buttons were obtained from five eyes of five patients collected following penetrating keratoplasty performed for visual rehabilitation. Corneal buttons were sectioned and analysed by immunofluorescent labelling with specific markers for macrophages, lymphocytes, dendritic cells and laminin.

Results
 Immunohistochemical markers for macrophages stained positively in the basal epithelium of the post-hydrops corneal sections. Lymphocytes were identified in the anterior- to mid-stroma. Presumed “resident” Langerhans cells were identified in the epithelium and anterior stroma. Unusually, dendritic cells were also identified in the endothelium of a cornea that developed neovascularisation post-hydrops. Laminin deposition was demonstrated in localised areas of the stroma, corresponding to the site of hydrops involvement.
Conclusions

Immunohistochemical analysis of post-hydrops corneas in keratoconus revealed extensive deposition of scar tissue and a chronic, inflammatory process with recruitment of immuno-inflammatory cells, including lymphocytes, macrophages and Langerhans antigen presenting cells. Unusually, dendritic cells were also observed in the endothelium in a cornea that developed neovascularisation. It appears that chronic inflammatory processes may persist when the acute phase of corneal hydrops appears to have clinically resolved which may influence success of corneal allograft surgery.
6.2 Introduction

As noted in earlier chapters, keratoconus is an ectatic disorder of the cornea, classically described as progressive and non-inflammatory – being characterised by central corneal thinning, corneal protrusion, and irregular myopic astigmatism.\textsuperscript{82} In New Zealand, approximately half of the penetrating keratoplasties performed each year are for the rehabilitation of visual impairment caused by this disease.\textsuperscript{16}

As highlighted in detail in Chapter 4, acute corneal hydrops is still a poorly-understood complication of keratoconus. The development of marked corneal oedema due to a break in Descemet’s membrane usually occurs in eyes with advanced thinning and ectasia, with an incidence of 2.4 – 3.0\% of eyes with keratoconus, typically causing a sudden further deterioration in vision.\textsuperscript{4,13,26}

Whilst acute hydrops in keratoconus usually self-resolves over 2-4 months,\textsuperscript{9,10} it is associated with severe epiphora, photophobia and pain, thereby rendering the affected, but typically otherwise healthy, young individuals to a poorly functioning state. Furthermore, the condition frequently leaves a vision-impairing scar, expediting the need for corneal transplantation to achieve visual rehabilitation. Significantly, penetrating keratoplasty post-hydrops is associated with a greater risk of failure due to increased likelihood of neovascularisation\textsuperscript{19} and reportedly higher risk of graft rejection.\textsuperscript{13,20}

There are limited pathophysiological investigations into acute hydrops in keratoconus. The prospective study by the author highlighted in the preceding
chapter assessed the clinical course of acute hydrops, and its microstructural changes, using in vivo confocal microscopy in ten patients with acute hydrops in keratoconus and demonstrated novel observations. Hyper-reflective round cells in the epithelium and stroma were exhibited in four of ten corneas. Elongated branching cells with small cell bodies were noted in the anterior stroma in two cases at 6 and 12 weeks respectively. Three months after presentation, both cases also exhibited unusual stromal cells with large speckled cell bodies and elongated branching cell processes. Both cases subsequently developed corneal neovascularisation.

In this follow up study, the aim was to identify the specific microstructural changes following acute hydrops in keratoconus with the assistance of immunohistochemistry in a subset of the original cohort.

6.3 Materials and methods

Subjects
Nine of the ten subjects with keratoconus-related acute hydrops highlighted in the preceding chapter went on to require penetrating keratoplasty for visual rehabilitation. A subset of five central corneal buttons obtained at the time of corneal transplantation was examined with the permission of the donors. Written informed consent was obtained from each subject and research ethics approval was obtained from the Northern X Regional Ethics committee prior to tissue use. All corneal buttons were between 7.5 mm to 8.5 mm in diameter. Corneas were stored and transported in New Zealand Eye Bank medium (2% FCS, 2 mM L-
glutamine, and 13 Anti–Anti in Eagle MEM) or New Zealand Eye Bank transport medium (additional 5% dextran in New Zealand Eye Bank medium).

**Table 7.1** Panel of immunohistochemical antibodies used in this study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Description</th>
<th>Dilution</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laminin</td>
<td>Rabbit anti-mouse polyclonal IgG</td>
<td>1:60</td>
<td>Sigma, L-9393</td>
</tr>
<tr>
<td>Langerin</td>
<td>Monoclonal mouse anti-human IgG2b</td>
<td>1:20</td>
<td>Leica Biosystems, NCL-LANGERIN</td>
</tr>
<tr>
<td>CD11c-cy5</td>
<td>Mouse anti-human IgG1</td>
<td>2:1</td>
<td>BD Pharmingen, 551077</td>
</tr>
<tr>
<td>CD11b-Alexa488</td>
<td>Rat anti-mouse IgG2b</td>
<td>2:1</td>
<td>BD Pharmingen, 557672</td>
</tr>
<tr>
<td>CD45-LCA</td>
<td>Monoclonal mouse anti-human, IgG1</td>
<td>1:4</td>
<td>Dako M0701</td>
</tr>
<tr>
<td>HLA-DR-FITC</td>
<td>Monoclonal mouse anti-human IgG&lt;sub&gt;2a&lt;/sub&gt; H+L</td>
<td>1:5</td>
<td>BD347363</td>
</tr>
<tr>
<td>Cy3</td>
<td>Goat anti-rabbit</td>
<td>1:400</td>
<td>Jackson ImmunoResearch, 115-165-003</td>
</tr>
<tr>
<td>Alexa 546</td>
<td>Goat anti-mouse IgG</td>
<td>1:1000</td>
<td>Molecular Probes, A-11003</td>
</tr>
</tbody>
</table>
**Section Preparation and Immunohistochemistry**

Tissue was fixed in 2.5% paraformaldehyde for 1 hour followed by 3 washes for 15 min each in phosphate buffered saline (PBS) prepared from tablets (BR14; OXOID, United Kingdom). After snap-freezing tissue in OCT mounting medium (Tissue-Tek; Sakura Finetek, Torrance, CA), tissue was sectioned in 20 µm steps.

Sections collected on slides were washed in 3 x 15min in 0.1M Tris Saline Buffer, pH 7.4. Slides were treated with 2mg/ml Testicular Hyaluronidase for 1hr at 37°C, followed by methanol at -20°C for 20 min, and 20 mM Glycine for 30 min at room temperature.

After applying 2% goat serum + 0.1% Trition X-100 for 30 min as treatment, slides were incubated with 1° antibody in 0.1% goat serum overnight. Slides were subsequently incubated with 2° antibody for 2 hours at room temperature in dark conditions.

Slides were then labelled with 4',6-diamidino-2-phenylindole (DAPI) for 10 min before sealing slides with coverslips. The details of the panel of antibodies used are presented in table 7.1.

**Histological labelling**

20 µm cryosections that were previously fixed in 2.5% paraformaldehyde were stained in haematoxylin and eosin (H&E).
**Image and statistical analysis**

Montaged images of full-width fluorescently labeled sections were collected using a fluorescence microscope with 20x and 40x lenses (Leica DR RA, Leica Microsystems, Heidelberg, Germany), via a digital camera (Nikon DS-5Mc; Nikon Corporation, Tokyo, Japan) connected to a desktop computer (Dell Computer Corporation, Austin, TX) running Windows Vista (Microsoft Corporation, Seattle, WA) and NIS-Elements BR Imaging software (Nikon Corporation).

Statistical analyses were performed on Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and values are expressed as mean ± standard deviations unless otherwise stated.

### 6.4 Results

**Patient information**

Of the five patients whose corneas were collected, two were male and three were female. The mean age was 20.8 ± 3.1 years (range 17 – 24 years) at time of onset of acute hydrops.

All five patients were of Maori or Pacific ethnicity. Three right eyes and two left eyes were involved. Details of the patients studied are outlined in greater detail in table 7.2.
Table 7.2  Demographic and clinical data: patients undergoing analysis of corneal buttons following penetrating keratoplasty for keratoconus related acute corneal hydrops.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Ethnicity</th>
<th>Eye</th>
<th>Time to PKP (months)</th>
<th>Presence of NV pre-PKP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>Female</td>
<td>Samoan</td>
<td>R</td>
<td>3</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>Male</td>
<td>Cook Island</td>
<td>L</td>
<td>4</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>Female</td>
<td>Samoan</td>
<td>R</td>
<td>4</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>Female</td>
<td>Maori</td>
<td>R</td>
<td>6</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>23</td>
<td>Male</td>
<td>Maori</td>
<td>L</td>
<td>10</td>
<td>No</td>
</tr>
</tbody>
</table>

NV = Neovascularisation; PKP = penetrating keratoplasty

The mean time to penetrating keratoplasty from presentation with acute hydrops was 5.4 ± 2.8 months (range 3 - 10 months). Further details regarding the patient characteristics and treatment of hydrops during the acute period have previously been published (see preceding chapter).\(^10\)

**Morphologic details**

A total of 450 sections were obtained with a mean of 92 sections per corneal button. Forty-six slides were made per cornea, with two sections on each slide. For each cornea, four sections were stained with H&E and the remaining sections were labeled with immunofluorescent antibodies.

Of the corneas that were stained with H&E, the majority demonstrated classic pathological signs of keratoconus including disruption of epithelial basement membrane, down-growth of epithelium into Bowman’s membrane, breaks in Bowman’s membrane, compaction of stromal fibrils – especially at site of hydrops,
concentrated areas of cell nuclei, and breaks in Descemet's membrane (figure 7.1a).

However, one of the corneas stained with H&E demonstrated severe stromal thickening with both inter-lamellar and intra-lamellar expansion. Keratocyte nuclei were evenly spread-out throughout the stroma and appeared more sparse than usual in post-hydrops corneas. The presence of pseudocysts was exhibited (figure 7.1b). In this cornea the epithelium remained uniform in thickness and was much thinner than other post-hydrops corneal sections. The same could be described of its Descemet's membrane and endothelium although it was difficult to be certain of this due to possible artifactual changes. This specific cornea sustained severe intrastromal and epithelial oedema *in vivo*, prior to transplant, that extended to the limbus for at least 120° hydrops with persistence of epithelial bullae on *in vivo* confocal microscopy despite clinical resolution of oedema (patient 2).
**Figure 7.1**

Haematoxylin and eosin- stained sections of post-hydrops corneas (figure on following page).

a. Representative section of typical post-hydrops cornea demonstrating downgrowth of epithelium into Bowman’s membrane (arrowhead), breaks in Bowman’s membrane (white arrow), compaction of stromal fibrils (encircled in white), concentrated areas of cell nuclei (encircled in white), and breaks in Descemet membrane (black arrow).

b. An atypical section demonstrating severe stromal thickening with both inter-lamellar and intra-lamellar expansion. The presence of pseudocysts is exhibited (black arrows).
Immunohistochemistry

One of the five corneas assessed stained positively with CD11b antibody – a marker for macrophages/monocytes (patient 1). This was visible throughout the basal epithelium as small round cells that fluoresce green (figure 7.2a, white arrows, and magnified view in insert). The same section overlaid with DAPI is presented in figure 7.2b.

In one cornea, the basal epithelium stained positively with langerin, a c-type lectin expressed by specific dendritic cell populations, including epithelial dendritic cells in the cornea. The anterior stroma of another cornea also stained positively with langerin. In both corneas, langerin stained scarcely and could only be seen in isolated areas of the basal epithelium or anterior stroma as fine fleck-like cells, fluorescing red (figure 7.3c).

Positive staining with HLA-DR-FITC was seen in the endothelium of three corneas, but most markedly in the cornea that developed neovascularization during the process of hydrops, as demonstrated in figure 2c and d (patient 2). Slightly linear, spindle-shaped cells in fluorescent green were seen in localized areas of the endothelium (figure 7.2c and d). HLA-DR is a cell receptor for human class II major histocompatibility complex (MHC) antigen, present on professional antigen-presenting cells (APCs), such as dendritic cells, B cells, Langerhans cells and macrophages.
Figure 7.2

Immunofluorescent staining (figure on following page) of various cellular populations demonstrated in post-hydrops keratoconic corneal sections included:

a. Positive staining of CD11b antibody – a marker for macrophages/monocytes, demonstrated throughout the basal epithelium (white arrows) and demonstrated in higher magnification in insert.

b. Section (a) overlapped with DAPI.

c. Positive staining of HLA-DR-FITC – a marker for antigen presenting cells, exhibited in the endothelium in of the cornea that developed neovascularization during the process of hydrops (red box).

d. Magnified image of HLA-DR-FITC$^+$ cells present in the endothelium (white arrows).

e. and f.

Positive staining with CD45 (LCA) antibody, a marker for lymphocytes, can be demonstrated to extend from the basal epithelium to mid stroma (e and f), and in the posterior stroma in certain sections (f), most prominently at sites of hydrops scarring. When overlapped with DAPI, concentrated nuclei can be seen in the same areas that stain positively with anti-CD45 (LCA), confirming an increase in cellular deposition.
There was remarkable staining with CD45 (LCA) antibody in three corneas. CD45 (LCA) labels the cell membranes of almost all leukocytes. However, CD45 is expressed less on mature granulocytes than lymphocytes (http://www.dako.com/dist/ar45/p109660/prod_products.htm, Dako Corporation, Denmark) and as such it signifies primarily lymphocytic deposition in this setting. This staining was demonstrated to extend from the basal epithelium to mid stroma, and in the posterior stroma, in certain sections, most prominently at sites of hydrops scarring (figure 7.2e and f).
When overlapped with DAPI, greater concentrations of nuclei were seen in the same areas that stained positively with anti-CD45 (LCA), confirming an increase in cellular deposition. The two representative corneal sections seen in figure 7.2e and f originated from the same corneal button as the H&E-stained section in figure 7.1b (patient 2).

Sections were also labeled with laminin and, as expected, highlighted the epithelial basal lamina, Bowman’s layer and Descemet’s membrane of all corneas. However, sections also stained positively in localized areas of the anterior to mid stroma in four of the five corneas (represented in figure 7.3a and b, and figure 7.4). When overlapped with DAPI, a slightly increased concentration of nuclei was demonstrated.

**Figure 7.3**

Immunofluorescent staining (figure on following page) of cellular and extracellular features demonstrated in post-hydrops keratoconic corneal sections.

a. and b. Corneal sections labeled with laminin, highlighting increased laminin deposition in localized areas of the anterior to mid stroma (fluorescent red). When overlapped with DAPI, increased cellular deposition is also demonstrated.

c. Epithelial Langerhans cells staining positive for langerin demonstrated sparsely in the basal epithelium (fluorescent red, white arrows).
Figure 7.4  High magnification 3D confocal microscopy of laminin deposition in the pre-Descemet’s membrane (DM) region following acute corneal hydrops. Laminin clearly defines the endothelial basement membrane (BM) as expected, but extensive laminin deposition is seen around the keratocytes of the posterior stroma (PS) immediately adjacent to DM as exhibited by the globular nature of the staining pattern. (Scale bar = 10um)
6.5 Discussion

To the author’s knowledge, this is the first reported immunohistochemical investigation of corneal hydrops in keratoconus. Earlier published laboratory studies in the literature of hydrops consist mainly of histological case reports.\textsuperscript{7,83,84} However, more recently, IVCM and other anterior segment imaging technology have expanded our understanding of the patho-physiological process of acute hydrops in keratoconus.\textsuperscript{17,85,86} Nonetheless, without immunohistochemical labeling, it is difficult to accurately interpret the ultrastructural changes beyond speculation. Furthermore, this laboratory study aimed to demonstrate the long-term microstructural sequelae post-hydrops in keratoconus that may be difficult to demonstrate \textit{in vivo} as the changes are subtle and beyond the resolution of \textit{in vivo} imaging modalities.

Perhaps the most important overall findings are the extensive inflammatory processes that take place in post-hydrops corneas. CD11b\textsuperscript{+} monocytic cells, APCs including Langerhans cells, and lymphocytes have been demonstrated throughout layers of the post-hydrops corneas. Interestingly, in recent years, a paradigm shift has taken place and the tenet that the human cornea was devoid of all bone marrow derived cellular elements has been largely refuted.

Independent research groups have identified the presence of CD11c\textsuperscript{+} CD11b\textsuperscript{-} Langerhans cells in the epithelium,\textsuperscript{87,88} CD45\textsuperscript{+} monocytic cells in the corneal stroma,\textsuperscript{89,90} and a separate, distinct, population of myeloid monocytic (CD11b\textsuperscript{+}) CD11c\textsuperscript{+} dendritic cells in the very anterior portions of the cornea stroma.\textsuperscript{89} In
addition, a population of CD14\(^+\) undifferentiated cells has been identified throughout the stroma\(^{87,89}\). Therefore the discovery of the presence of some of these cells post-hydrops is perhaps not surprising.

The current study demonstrated the presence of CD11b\(^+\) monocytic cells, likely macrophages, in the basal epithelium of the cornea post-hydrops. This cell type is now understood to be part of the resident myeloid cell population of the normal corneal stroma. In the context of insults to the cornea, macrophage infiltration of the corneal stroma, following infiltration by neutrophils recruited from the limbal cell population is part of the inflammatory cascade - usually in response to a breach of the blood-aqueous barrier.\(^{91-93}\) The presence of macrophages in the basal epithelium in the current study is likely to be representative of inflammatory recruitment of macrophages, as resident monocytic cells have not previously been found in the corneal epithelium. Interestingly, the cornea in which this cell population was identified did not have hydrops that extended to involve the limbus.

A chronic inflammatory process in this setting is further supported by the presence of lymphocytic deposition in the anterior to mid stroma, as demonstrated by positive labeling with CD45 (LCA) antibody.

The presence of professional antigen presenting cells in the endothelium is a novel finding and in this case is likely to be a population recruited following hydrops. Resident cell populations have not previously been reported in the corneal endothelium. APCs prime T cells by presenting antigens in the groove of MHC class II along with the requisite co-stimulatory signaling.\(^{94}\)
The uniqueness of the bone marrow-derived resident APCs of the cornea lie in the fact that they are universally both MHC class II- and co-stimulatory factor-negative. The cells identified in the current study stained positively for HLA-DR, which is a cell receptor for MHC class II. The labeling was positive only in the cornea which sustained severe hydrops that extended to the limbus and subsequently developed neovascularisation.

We postulate that the presence of these cells within the endothelium may be directly associated with the development of neovascularisation. The leukocytes that mediate these immuno-inflammatory responses are derived from the (limbal) intravascular compartment and the greater the surface area the blood vessels have in contact with the tissue, the more pronounced the effect of the inflammatory process. Interestingly, dendritic cells in the endothelium were never visualised in the IVCM component of this study (see Chapter 5). The atypical nature of this particular cornea and the severity of the hydrops process can be further demonstrated on the H&E-stained histology section as seen in figure 6.1b, exhibiting the gross stromal thickening with intra- and inter-lamellar expansion, the persistence of pseudocysts, and the thinning of the epithelium.

In contrast, the presence of langerin-positive dendritic cells in the corneal epithelium and anterior stroma may not signify an alteration in cell population post-hydrops as resident dendritic cells have previously been demonstrated in the corneal basal epithelium. Further support of this is that these cells are located both paracentrally and very sparsely. This is consistent with the observations of
Mayer et al. in a population of post-herpes keratitis, post-graft rejection, and keratoconic (hydrops not specified) corneas following penetrating keratoplasty.\textsuperscript{97}

Laminins are proteins commonly found in the basal lamina of major organs. In the cornea, the epithelial basement membrane, Bowman’s layer and Descemet’s membrane are composed of laminins, primarily laminin-1 and -5. It is now well-established that initial repair and subsequent fibrosis and remodeling all involve laminin as a supportive protein,\textsuperscript{98-100} and it is one of the first extracellular matrix proteins deposited by keratinocytes following wounding.\textsuperscript{101} It has a role not only in cell adhesion in the basement membrane, but also promotion of epithelial motility during repair.\textsuperscript{102} Interestingly, laminin deposition following trauma may be slightly increased in keratoconic corneas compared to non-keratoconic corneas.\textsuperscript{103} In the present study, laminin deposition has been demonstrated in very localized areas of the stroma, more prominently in the anterior to mid stroma, representative of a fibrotic process.

Increased laminin deposition in the epithelial basement membrane and gaps in Bowman’s layer and anterior stroma has previously been described in scarred keratoconic corneas (a history of hydrops not indicated).\textsuperscript{104} Sparse laminin deposition has also been documented in association with sub-epithelial fibrosis in bullous keratopathy,\textsuperscript{105} a process with some similarities to acute corneal hydrops. Unfortunately, in the current study we were unable to identify the stromal cells with large speckled cell bodies and elongated branching cell processes seen on IVCM in two corneas that developed neovascularisation (only one of which was analysed in this study). While they were postulated to be specific antigen-
presenting cells, we have not been able to isolate them *ex vivo*, despite the fact that other investigators have found higher dendritic cell density *ex vivo* with immunohistochemistry than with IVCM.\textsuperscript{97} There are several possible reasons for this. Firstly, it is possible that the cells were no longer present at the site following the *in vivo* investigation as the disease continued to resolve. Secondly, in both *in vivo* and *ex vivo* analyses the areas exhibiting these cells are relatively small and therefore the sampled regions may not overlap. Thirdly, the process of trephination and tissue processing may have destroyed the cells. Finally, appropriate immunohistochemical antibodies to identify the cells may not have been utilised.

In conclusion, this study demonstrated, with the assistance of immunohistochemistry, the pronounced chronic inflammatory process that takes place during and following acute hydrops in keratoconus. Even for corneas that did not develop oedema extending to the limbus, inflammatory cells such as macrophages and lymphocytes are recruited. Furthermore, antigen-presenting cells were identified in the endothelium of a cornea with neovascularisation. This study also confirmed the presence of resident corneal dendritic cells in the epithelium and anterior stroma that are unlikely to be related to the hydrops process. The authors hypothesise that there are further, as-yet-unidentified, leukocytic populations present in the post-hydrops cornea. Given that the majority of eyes post-hydrops will require corneal transplantation for visual rehabilitation, the prominence of the inflammatory process with recruitment of immunoinflammatory cell populations may predispose these corneas to a significantly increased risk of allograft rejection post-transplantation. There is, therefore,
preliminary evidence to suggest that more aggressive topical immunosuppressive treatment of all corneas during acute hydrops may be beneficial. Further studies are required to identify other cell populations and to confirm this hypothesis.
Chapter 8

Keratoconus and keratoconus

surgery-related case studies
8.1 Long-term corneal microstructural changes following epikeratophakia for aphakia: *in vivo* confocal microscopic analysis of an uncommon technique previously employed in keratoconus

8.1.1 Abstract

The unilateral epikeratophakic eye of a 20-year-old female with a history of congenital cataracts was examined using laser scanning *in vivo* confocal microscopy 17-years following transplantation. *In vivo* confocal microscopy demonstrated a reduced keratocyte density in both the grafted lenticule and the host stroma, with unusual elongated and tortuous hyper-reflective branching structures in the anterior stroma of the host cornea. The sub-basal nerve plexus was present in the lenticule, although with a reduced nerve density. The host endothelium exhibited an appearance similar to that observed in Fuch's endothelial dystrophy. Dramatic microstructural changes were observed in almost all layers of the cornea 17-years following epikeratophakia. Though no longer performed as routine practice, *in vivo* confocal microscopy examination of epikeratophakia has nevertheless offered us fascinating insight into the potential corneal adaptations at a cellular level.
8.1.2 Introduction

Essentially, epikeratophakia is a surgical procedure in which a stromal lenticule of donor human corneal tissue is sutured onto the anterior surface of the recipient cornea denuded of epithelium in order to change its anterior curvature and refractive properties. Although first introduced for the rehabilitation of vision in aphakia as an alternative to secondary implantation of intraocular lenses, the commonest indication for epikeratophakia was advanced keratoconus when other treatment options, such as corrective visual aids and penetrating keratoplasty, were not tolerated or appropriate. To a lesser extent, epikeratophakia has been indicated in myopia and hyperopia for similar reasons. Because it is a form of onlay lamellar keratoplasty, it has many of the advantages of lamellar keratoplasty, being an extraocular procedure, such as preservation of host endothelium, elimination of the risk of endothelial rejection and traumatic dehiscence of the globe, as well as being potentially reversible, and possibly less technically challenging.

We present an interesting case of epikeratophakia 17 years following initial transplant, with a focus on corneal microstructure imaged by in vivo confocal microscopy.

8.1.3 Methods

Subject recruitment and assessment
A healthy 20-year old female presented to the ophthalmology clinic for exploration of treatment options for her right eye (OD). She had a history of congenital cataracts, for which the left eye (OS) was treated by extracapsular cataract extraction and intraocular lens implantation at the age of 18 months. The right eye (OD) was treated by intracapsular cataract extraction and epikeratophakia at 3 years of age. All ophthalmic procedures had been performed in the USA and further surgical details were unavailable. Despite surgical intervention the right eye became amblyopic.

Following a detailed explanation and acquisition of informed consent, slit-lamp biomicroscopy and laser scanning in vivo confocal microscopy (Heidelberg Retina Tomograph II [HRT II] Rostock Corneal Module [RCM]; Heidelberg Engineering GmbH, Heidelberg, Germany) were performed on both eyes. The particulars of the RCM and the images it produces have been described in detail elsewhere.111

Image analysis

For the left cornea, 3 images were selected from each of the following levels: basal epithelium, sub-basal nerve plexus, anterior stroma, mid stroma, posterior stroma and endothelium. For the right cornea, three images were selected from each of the following levels: basal epithelium of lenticule, sub-basal nerve plexus of lenticule, stroma immediately posterior to Bowman’s layer of the lenticule, and lenticule stroma immediately anterior to the interface, the interface between lenticule and host cornea, anterior stroma of the host cornea, mid-stroma of the host cornea, posterior-stroma of host cornea, and endothelium. Anterior stroma
was defined as the first 3 clear images (without motion blur or compression lines) immediately posterior to Bowman’s layer; posterior stroma was defined as the first 3 clear images immediately anterior to Descemet’s membrane, and mid-stroma was defined as 3 images equidistant from Bowman’s layer and Descemet’s membrane.

Measurements were performed using a calliper tool (analySIS 3.1, Soft Imaging System, Münster, Germany). A standard central counting frame size of 300 µm x 300 µm was used. The method of cell counting and measurement of sub-basal nerve density have also previously been described in detail.\textsuperscript{111}

**Statistical analysis**

All values were entered into a Microsoft Excel database and subsequently imported into statistical software for analysis. Statistical analysis was performed in SPSS Version 12 for Windows (Chicago, IL, USA). Paired-Samples T-test was used to compare values between two groups. All tests were two-tailed and a P value of less than 0.05 was considered statistically significant.

**8.1.4 Results**

The subject’s best spectacle-corrected visual acuity (BSCVA) was 1/60 OD and 6/6 OS. Automated refraction was -5.25 D / -2.75 D x 147° OD, and +0.75 D / -0.75 D x 23° OS. Slit-lamp biomicroscopy revealed a 7.1 mm diameter, inferotemporally-decentred lenticule on the host cornea of the right eye (figure 8.1.1a). Both lenticule and host cornea were clear barring occasional ghost blood
vessels visible in the interface (figure 8.1.1c). The corneal endothelium had a beaten metal appearance (figure 8.1.1b and d). The pupil was oval-shaped and decentred superonasally and the eye was aphakic. The left cornea appeared clinically normal and there was a superior peripheral iridotomy and a posterior chamber intraocular lens. The remainder of the ocular examination was unremarkable.

*In vivo* confocal microscopy of the left eye (OS) revealed essentially normal corneal microstructure (figures 8.1.2e-f, 8.1.3d-f).

The layers of the right lenticule (OD) could be easily distinguished as basal epithelium (figure 8.1.2a), sub-basal nerve plexus (figure 8.1.2b), and a partial-thickness stroma (figures 8.1.2c). The host-lenticule interface was an amorphous homogenous layer with appearances consistent with Bowman's layer (figure 8.1.2d). The remaining host stroma appeared morphologically normal (figures 8.1.3b). The host endothelium exhibited guttae and endothelial cells appeared enlarged and irregularly shaped (figure 8.1.3c).
Figure 8.1.1 Photographs of the right eye. a: Low magnification image of right eye depicting epikeratophakic cornea and displaced pupil; b: Reduction of cell density and beaten-metal appearance of the endothelium; c: Fine blood vessels present at the lenticule–host interface; d: Slitlamp photography of lenticule (white arrows) and host cornea (red arrows) separated by interface.
Figure 8.1.2 In vivo confocal microscopy comparing the grafted lenticule (right eye) with the normal counterparts in the left eye: basal epithelium of lenticule (a, e); sub-basal nerve plexus of lenticule (b, f); lenticule stroma immediately posterior to Bowman layer (c); graft–host interface (d).
Mean cell densities in the lenticule and host cornea of the right eye are presented in table 8.1.1. Anterior stromal and endothelial cell densities for the right host cornea could not be measured due to the indistinct cell borders. The lenticular sub-basal nerve plexus (figure 8.1.2b) had a significantly lower density compared to the contralateral cornea (p=0.014). There was no significant difference between the basal epithelial density of the left cornea and that of the lenticule (p=0.874). However, the mean keratocyte densities of the right lenticule and host stroma were significantly lower compared to those of the left stroma (Table 8.1.1).
Table 8.1.1 Mean cell densities and sub-basal nerve densities of the epikeratophakic cornea (right eye) and the left cornea.

<table>
<thead>
<tr>
<th></th>
<th>Right eye</th>
<th>Left eye</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal epithelium (cells/mm²)</td>
<td>5313 ± 159.1</td>
<td>5475 ± 989.9</td>
<td>0.874</td>
</tr>
<tr>
<td>Sub-basal nerve density (mm/mm²)</td>
<td>5.01 ± 0.50</td>
<td>8.34 ± 0.30</td>
<td>0.014</td>
</tr>
<tr>
<td>Lenticule stroma immediately posterior to Bowman’s layer (cells/mm²)</td>
<td>296 ± 92.6</td>
<td>N/A</td>
<td>(Compared to left anterior stroma) 0.032</td>
</tr>
<tr>
<td>Lenticule stroma immediately anterior to interface (cells/mm²)</td>
<td>178 ± 95.0</td>
<td>N/A</td>
<td>(Compared to left mid-stroma) 0.049</td>
</tr>
<tr>
<td>Anterior stroma (cells/mm²)</td>
<td>N/A</td>
<td>607 ± 218.9</td>
<td>N/A</td>
</tr>
<tr>
<td>Mid-stroma (cells/mm²)</td>
<td>208 ± 7.0</td>
<td>483 ± 38.2</td>
<td>0.005</td>
</tr>
<tr>
<td>Posterior stroma (cells/mm²)</td>
<td>246 ± 89.9</td>
<td>458 ± 38.2</td>
<td>0.074</td>
</tr>
<tr>
<td>Endothelium (cells/mm²)</td>
<td>N/A</td>
<td>3392 ± 72.2</td>
<td>N/A</td>
</tr>
</tbody>
</table>
**Figure 8.1.3** *In vivo* confocal microscopy comparing the host cornea (right eye) with its normal counterparts in the left eye: anterior stroma (a, d); mid-stroma (b, e); endothelium (c, f).

![Confocal Microscopy Images](image-url)
8.1.5 Discussion

As a result of advances in surgical techniques, it is becoming rarer to see cases of epikeratophakia, yet the technique offers a fascinating insight into the modification of corneal structure and its effects at a cellular level.

This case report makes several noteworthy observations but in turn also raises a few questions. Firstly, sub-basal nerves were present in the lenticule. The question thus begs: where did they come from? As the sub-basal nerve plexus of the donor lenticule would have degenerated long before transplantation these nerves must be derived from the host’s sub-basal nerve plexus, yet no sub-basal nerves were identified in the host Bowman’s layer. The lenticule itself was unlikely to have survived so long in such a healthy state without the protective and trophic influences of corneal innervation.\textsuperscript{112,113} Unsurprisingly, the nerve fibre density was reduced significantly in concordance with a previous study that reported reduced sub-basal nerve density up to 40 years following penetrating keratoplasty\textsuperscript{111} – in both procedures a circumferential incision is made in the recipient cornea. Even though in epikeratophakia this incision is usually to a depth of less than 150 um,\textsuperscript{114} one must presume that all sub-basal nerves are severed, and those more central to the incision removed during preparation of the recipient cornea. Yet those left in the periphery of the recipient were able to make their way into the Bowman’s layer of the lenticule. Whether this reinnervation occurred through the host Bowman’s layer or through the keratectomy site is uncertain. However the presence of sub-basal nerves in the lenticule, but not in the host, suggests that there are epithelial or sub-epithelial factors required to maintain the sub-basal nerve plexus. Of course it is possible, though less likely, that sub-basal nerves were present in the
host cornea during examination but were simply not visualised on in vivo confocal microscopy. A histopathologic examination would be required to confirm our speculations.

It was interesting to observe that keratocyte density was significantly lower in both the lenticule and host stroma compared to the contralateral corneal stroma. Most studies analysing cell densities following penetrating keratoplasty have reported significantly lower keratocyte densities in the post-transplantation cornea compared to the non-transplanted cornea,\textsuperscript{111,115} therefore a reduced keratocyte density in the lenticule is unsurprising. In fact, one may have expected an even lower keratocyte density given that, as part of the process of preparing the donor lenticule, the tissue is frozen and lathed prior to grafting. Stromal keratocytes have been shown to be irreversibly damaged by freezing.\textsuperscript{116} Furthermore, as keratocytes are unable to cross Bowman’s layer and must move centrally from the peripheral cornea, there is a much narrower peripheral area for the host keratocytes to access the lenticule compared to that in penetrating keratoplasty. What is surprising, however, is that the current case also noted decreased keratocyte density in the host stroma. Following penetrating keratoplasty, donor cells initially persist in the donor cornea but are slowly replaced by host keratocytes.\textsuperscript{117} It is possible that as host keratocytes replaced the lenticule keratocytes, the same density failed to be regenerated in the host stroma. Alternatively, there may be increased apoptosis in both the host and transplanted stroma in view of the fact that keratocytes in transplanted corneas are known to undergo increased apoptosis compared to the normal cornea.\textsuperscript{118}
The unusual appearance of the anterior stroma of the host cornea (figure 8.1.3a) has not previously been reported. The authors postulate that the unusual appearance represents abnormal keratocytes that have transformed in attempt to extend anteriorly into the lenticule.

It was also remarkable to observe the strawberry-like endothelial appearance similar to that observed in Fuch’s endothelial dystrophy. One explanation for this is the history of intracapsular cataract extraction in childhood and associated reduced endothelial cell density, and the subsequent development of mild aphakic bullous keratopathy. The need to serve a thicker cornea may also attribute to the cellular change.

In conclusion, the microstructural changes of the cornea following epikeratophakia have been further delineated by in vivo confocal microscopy. Though no longer performed as routine practice, this case of epikeratophakia has provided us with an understanding about the potential corneal cellular adaptations not observed with routine keratoplasties and unimaginable prior to the advent of in vivo confocal microscopy.
8.2 Clinical and histological manifestations of an extreme Descemet’s Membrane tear in keratoconus

8.2.1 Abstract

Acute hydrops is a poorly understood complication of keratoconus associated with high morbidity in an otherwise healthy and young population. It typically results in a vision-impairing scar that necessitates corneal transplantation.

A case of unilateral acute hydrops in a 20-year-old male with keratoconus is reported. The acute hydrops was associated with a large Descemet’s membrane tear that persisted despite clinical resolution of corneal oedema. It resulted in a flat cornea (maximum keratometry 36.2 D), associated with a large, central corneal stromal scar that required penetrating keratoplasty for visual rehabilitation. This case illustrates the possible extent and patterns of Descemet’s tear during acute hydrops in keratoconus. Despite the dimensions of the tear, resolution of corneal oedema still occurred within 6 weeks, demonstrating the ability of the corneal endothelium to remodel and function with deficient Descemet’s membrane. We postulate that the size of the tear is directly related to the size of the final corneal scar. Further prospective trials are required to confirm this hypothesis.
8.2.2 Introduction

A tear or split in Descemet’s membrane (DM) resulting in acute corneal hydrops is a well-recognised complication of corneal ectasias and keratoconus in particular. The tear is usually relatively small and localised, at the site of the most advanced thinning. A case is presented of an unusually large Descemet’s tear in a cornea with keratoconus.

A 20-year-old New Zealand European male with keratoconus presented with a 6-week history of mild discomfort, watering, “whitening” of his cornea, and reduced vision in his right eye suggestive of acute corneal hydrops.

Keratoconus was diagnosed at the age of 12 years with long term correction with rigid gas permeable contact lenses. There was a strong family history of keratoconus (father and two brothers) and he admitted to habitual eye-rubbing. His past medical history was significant for atopic rhinitis and asthma.

On examination unaided vision was counting fingers in each eye, improving to 6/48 with pinhole right eye, and 6/9 left eye with contact lens. There was advanced corneal oedema in the right eye associated with an extensive infero-central DM rupture. The left eye had signs of moderately-severe keratoconus. The rest of the ocular examination was unremarkable.
He was treated with topical lubricants and g. Prednisolone acetate 1% QDS to the right eye. Over the next 6 weeks the stromal and epithelial oedema gradually resolved leaving large central corneal scarring and flattening.

**Figure 8.2.1**

A. Clinical photograph of cornea at 6-week post initial presentation demonstrating torn edges of Descemet's membrane (DM) (black arrows).
B. Dark field microscopy image of excised button, showing torn edges of DM (black arrows).
C. Magnified dark field microscopy image of torn DM (black arrows), with folds of DM surrounding the torn DM edges.
D. Montage of environmental scanning electron microscopic (ESEM) images of a torn DM edge with surrounding DM folds (scale bar = 200 um).
Interestingly, a large DM defect was still present (figure 8.2.1a, arrows). Orbscan corneal topography OD revealed 6.6 dioptres (D) of astigmatism at 147° with a Max-K of 36.2 D and Min-K of 29.6 D following resolution of hydrops. In comparison the left eye had simulated Max-K of 54.5 D and Min-K of 50.3 D (see figures 8.2.2 and 8.2.3).

**Figure 8.2.2** Orbscan tomography of the right eye at 11-months post- acute hydrops

Subsequently the patient underwent penetrating keratoplasty. Due to the unusual size and persistence of the DM tear consent was obtained for histological and immunohistochemical analyses of the corneal button. Following surgery the patient made an uneventful recovery, regaining vision of 6/15 unaided at 3-
months, with a post-operative subjective refraction of +4.00/-3.00 X 120, providing 6/9.

**Figure 8.2.3** Orbscan tomography of the left, non-hydrops eye.

The unusual extent of the DM defect is highlighted by the dark field images of the excised corneal tissue (figure 1b and c). The corneal button was prepared for histological investigation. Haematoxylin and eosin (H&E) staining was performed on 5 µm sections (Figure 8.2.4 and 8.2.5c). Antigen retrieval was performed on the fixed tissue to allow adequate immunohistochemical staining with laminin to reveal basement membranes (primary antibody), Cy3 (secondary antibody) and labelled with 4',6-diamidino-2-phenylindole (DAPI) to reveal cell nuclei. Fixatives were removed prior to viewing on an environmental scanning electron microscope.
Figure 8.2.4 H&E-stained section of excised corneal button demonstrating absence DM centrally with the torn edges of DM retracted into scrolls on each side with further folds of DM on the right-hand-side (black frame).
The H&E-stained section of the corneal button (figure 8.2.4) demonstrates the five layers of the cornea throughout the majority of the section. However, centrally, DM is absent due to the torn DM, the edges of which are visibly retracted and curled anteriorly, creating a scroll at each end (figure 8.2.5c, black arrows). Retracted folds of DM were readily visualised (figure 8.2.5c, white arrowheads). The folded DM edge is better demonstrated on the high-powered IHC and ESEM sections (figures 8.2.1d, 8.2.5a, b, d-f). The area of absent DM measured approximately 1400 µm x 500 µm.

Figure 8.2.5

a. An ESEM image of a torn edge of DM, curled into itself.

b. An image of immunohistochemically-stained section demonstrating the retracted and curled DM edge.

c. H&E staining of the central portion of the button shows the major geographical features of the Descemet rupture.

d, f. Folds of retracted DM as imaged by ESEM.

e. A fold of retracted DM as imaged after immunohistochemical staining.
8.2.3 Discussion

Acute corneal hydrops in keratoconus usually resolves spontaneously over two to four months,\textsuperscript{9,10} resulting in a central scar that typically necessitates corneal transplantation to achieve visual rehabilitation (see chapter 4 and 5). The resolution of hydrops is thought to occur after at least partial re-attachment of DM and enlargement and migration of adjacent endothelial cells to cover the defect.\textsuperscript{8,120,121}

This case is particularly unusual in that clinical resolution of acute hydrops had taken place despite minimal closure of Descemet’s tear and a large persistent DM defect. Although not visualised clearly on our imaging, it is obviously presumed that the endothelial cells are responsible for clearing the corneal oedema, and this case demonstrates that endothelial cells can still function well with an incomplete DM. This case also beautifully illustrates the various patterns that develop in the torn, detached edges of DM. Persistent scrolls and ridges are demonstrated in this study \textit{ex vivo}. Another study has attempted to identify various patterns of DM detachment in acute hydrops \textit{in vivo} with anterior segment optical coherence tomography (AS-OCT) and echoes our observations:\textsuperscript{8} detachment with break and rolled ends, detachment with break and flat ends, and detachment with no break. However, the authors acknowledged that because AS-OCT scans are taken at 45° intervals, a small planar break could have been missed, as detachment without break would question our present understanding of the mechanism of hydrops development.
There is little current understanding of the biomechanical process that leads to the different patterns of DM detachment in acute hydrops. One could hypothesise that the different patterns of detachment may be related to the extent of the tear and the severity of oedema and consequently its duration.

The extreme corneal flattening following resolution of hydrops demonstrated on topography is postulated to be associated with the extent of DM tear. As the oedema resolves, stromal tissue becomes compacted with deposition of scar tissue. Therefore, the size of the resultant scar is likely to be related to the size of DM defect. Corneal flattening post-hydrops frequently leads to improved contact lens fitting. However, central corneal scarring is the primary reason for the requirement of corneal transplantation following hydrops resolution. Theoretically, if the size of the scar can be reduced by modifying the size of the tear, the necessity for corneal transplantation following hydrops could also be reduced.

This case has highlighted several fascinating factors in keratoconus-related acute hydrops that are still poorly understood. Further prospective studies into endothelial function in the absence of intact DM, and the relationship between DM tear, duration of oedema and corneal scar size, may lead to advances in the management of this disease.
References from Section II


95. Dana R. Comparison of topical interleukin-1 vs tumor necrosis factor-alpha blockade with corticosteroid therapy on murine corneal inflammation,


Section III

Studies of Intraocular Pressure in Keratoconus
Chapter 9

*Intraocular pressure elevation in subjects with keratoconus that have undergone corneal transplantation*
9.1 Abstract

Purpose
To determine the incidence of post-keratoplasty intraocular pressure (IOP) elevation in eyes of subjects with keratoconus and establish the relationship between IOP and corticosteroid administration in this population.

Method
Following strict inclusion/exclusion criteria a retrospective analysis was performed on a consecutive series of penetrating keratoplasties performed for keratoconus observing a standardised surgical and post-operative regimen in Auckland, New Zealand. Patient demographics, ocular, medical, and family history and per-operative and post-operative data were recorded until 12 months post-keratoplasty.

Results
Fifty-seven eyes of 48 patients were included: 31% were New Zealand Europeans, 42% Pacific peoples, 15% Maori, 12% other. Eighteen eyes (32%) of 17 patients (35%) exhibited elevated IOP and 12 (21%) of eyes exhibited moderate to severe elevation of IOP. IOP elevation occurred 3 to 6-months post-keratoplasty in 78% of eyes. Elevated IOP was significantly less common in Maori and Pacific peoples (p=0.02). All eyes except one required reduction/cessation of corticosteroids in order to normalise IOP.

Conclusions
The incidence of presumed steroid-related post-keratoplasty IOP elevation, in 35% of subjects with keratoconus, is markedly higher in this New Zealand study than previously reported in US and UK studies. Further clinical and genetic analysis of associations between keratoconus, steroid-induced IOP elevation and glaucoma might improve our current understanding of this condition.
9.2 Introduction

Intraocular pressure (IOP) elevation is a known complication of topical corticosteroid therapy and was first described by Francois in 1954.\textsuperscript{1} Those who are likely to respond generally do so within a few weeks of continual steroid administration.\textsuperscript{1} Steroid-induced pressure response in children tends to be more prevalent and rapid than adults.\textsuperscript{2} In both adults and children, however, IOP typically returns to normal following cessation of steroid therapy.

Post-penetrating keratoplasty intraocular pressure control is a major concern for ophthalmic surgeons. Not only can prolonged IOP elevation damage the optic nerve,\textsuperscript{3} it can be harmful to the donor endothelium and ultimately affect the success of the corneal graft.\textsuperscript{4} It is also among the more difficult of the glaucomas to monitor due to the effect of significant astigmatism on accurate applanation measurement of IOP, particularly in the early post-operative period.\textsuperscript{5} Treatment of this elevated IOP is also challenging, as prolonged topical corticosteroid therapy is an inherent part of post-keratoplasty management.

In Australasia, keratoconus is the most common indication for penetrating keratoplasty.\textsuperscript{6,7} Studies that have examined elevation of IOP following penetrating keratoplasty have reported the incidence to be in the range of 10 – 35\%,\textsuperscript{3,8-11} however, keratoconus subjects have been noted to be among the least at risk to encounter this complication.\textsuperscript{3,12,13} Yet, in contradistinction, from clinical experience we suspected that a higher proportion of the keratoconus population in New Zealand experienced elevated IOP post-penetrating keratoplasty than previously
reported in the world literature. The current study was therefore undertaken to
determine the incidence of post-penetrating keratoplasty IOP elevation in
keratoconus as well as to establish the relationship between IOP and topical
corticosteroid administration in this population.

9.3 Methods

Patient selection
This study used a retrospective study design to assess subjects referred for
penetrating keratoplasty for keratoconus. Analysis was restricted to the records of
all penetrating keratoplasties performed by one corneal sub-specialist surgeon
(CNJM) for keratoconus in the period of January 2000 to March 2008 at the
Department of Ophthalmology, Auckland City Hospital and Eye Institute,
Auckland, New Zealand. A standardised assessment, surgical technique and post-
operative topical corticosteroid regime was used throughout the study period.

The exclusion criteria included: deep anterior lamellar keratoplasty, repeat
penetrating keratoplasty, any previous intraocular surgery, history of glaucoma,
any additional surgery within the follow-up period, and tertiary referrals from
outside the region who were unable to attend regular local follow-up. Three
patients who were lost to follow-up during the study period were also excluded.

Patient demographic information, ocular and medical history including any
previous use of topical or systemic corticosteroids, and family history of
keratoconus and/or glaucoma were recorded. Operative information recorded
included: endothelial density of donor cornea, type of sutures used, and intra-operative drug administration. Post-operative antibiotic and corticosteroid regime was standardised. Post-operative records were followed to 12 months post-keratoplasty for all patients and those (8 subjects) who had not reached this point of follow-up were excluded.

IOP measurements by Goldmann’s tonometer were taken routinely for each visit and were recorded for each of the following visits (or closest thereof): pre-operative assessment, 1-week post-operatively, 1-month, 3-months, 6-months, 9-months, and 12-months. Elevated IOP prior to 1-week post-surgery was excluded in order to avoid inadvertently including viscoelastic-related IOP elevation.

For the purpose of analysis, normal intraocular pressure was defined as 10 – 21 mmHg. Elevated IOP between 22 – 25 mmHg was considered “mild”, 26 – 30 mmHg “moderate” and measurements >30 mmHg were considered “severe” IOP elevation.

Any interventions, e.g. reduction in frequency/potency of corticosteroids or introduction of topical ocular hypotensives, in response to elevation of IOP rise and were recorded. The subsequent change in IOP in response to these interventions was noted. The total number of drops (cumulative dose) of topical corticosteroid administered during the entire postoperative period was determined from each individual patient record. Corneal topography with central corneal thickness measurements was also recorded in the post-operative period.
**Surgical technique and post-operative management**

Penetrating keratoplasty was performed using a Barron-Hessberg suction trephine system (Barron Precision Instruments, L.L.C., Michigan, USA). The donor material was excised from the endothelial side by using a Barron-Hessberg donor punch system (Barron Precision Instruments, L.L.C., Michigan, USA) and all donor tissue was sized 0.25 mm larger in trephine diameter than the host (excepting one case that was deliberately over-sized by 0.50 mm). The standard suturing technique used 12 interrupted 10/0 nylon sutures and an anti-torque continuous 11/0 nylon except when significant peripheral corneal neovascularisation was encountered – in which setting 16 interrupted 10/0 nylon sutures were utilised. In all eyes, a viscoelastic substance (Healon, Advanced Medical Optics, Inc. California, USA) was used to form the anterior chamber and protect the endothelium during surgery. Just prior to completion of surgery, this material was aspirated or washed out of anterior chamber and the anterior chamber was completely reformed with balanced salt solution and the water-tightness of the wound confirmed.

All patients received subconjunctival dexamethasone sodium phosphate (2 mg in 0.5 mL) and Cephazolin (100 mg in 0.5 mL) injections at the end of the procedure. All subjects were prescribed three doses of oral acetazolamide 250 mg in the first 18-24 hours post surgery.

Post-operative management included g. Chloramphenicol four times a day (Chlorsig, Sigma Pharmaceuticals) for 2-4 weeks and Prednisolone acetate 1% drops (Predforte, Alcon Laboratories) at approximately 2-hourly intervals during waking hours for the initial 7 post-operative days, then four times a day for six
months, reducing stepwise to once per day for months 9-12. Eight patients were subsequently placed on g. Dexamethasone 0.1% to reduce medication cost (g. Prednisolone acetate 1% is not a government-subsidised drug in New Zealand whereas g. Dexamethasone 0.1% is subsidised) or g. Fluoromethalone 0.1% if a less potent corticosteroid was required in the context of a steroid-related IOP elevation to g. Prednisolone acetate. The cumulative number of corticosteroid drops applied per patient was counted based on prescribed regime for the 12-month follow-up.

**Statistical Analysis**

Statistical analyses were performed in conjunction with a professional biomedical statistician. In order to investigate factors related to the occurrence of raised IOP post operation a generalised mixed model was fitted to allow adjustment for the within person correlation for those who had 2 eyes operated on. The binary outcome of elevated IOP or not was used with the logit link function and age, gender, and ethnicity as explanatory variables. Pre-operative topical steroid use, pre-operative systemic steroid use, pre-operative IOP, family history of keratoconus and post-operative central corneal thickness were also used as explanatory variables.

**9.4 Results**

In a largely tertiary referral practice, a total of 269 keratoplasties were performed by the one surgeon over the 8-year period, including 257 penetrating and 12 deep lamellar keratoplasties. Of the penetrating keratoplasties, 87 were performed for
keratoconus; 64 for regraft purposes; 36 for keratitis; 22 for pseudophakic bullous keratopathy; 20 for corneal dystrophies; 12 for trauma and 16 were conducted for other indications. Of the 89 primary penetrating keratoplasties conducted for keratoconus, 17 were excluded as they had not reached the 12-months post-surgical follow-up period; three were excluded due to previous intraocular procedures; one was excluded because the penetrating keratoplasty was done in conjunction with vitrectomy; and nine were either transferred back to the referring centre or lost to follow-up.

Table 9.1  Post-keratoplasty pressure elevation over 12-month follow-up in keratoconus

<table>
<thead>
<tr>
<th>Number of eyes</th>
<th>1-week</th>
<th>1-month</th>
<th>3-month</th>
<th>6-month</th>
<th>9-month</th>
<th>12-month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild (22-26 mmHg)</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Moderate (27-31 mmHg)</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Severe (&gt;31 mmHg)</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Therefore 57 eyes of 48 patients who met the exclusion and inclusion criteria were included in this study. The male-to-female ratio was 29:19. Right eye to left eye ratio was 22:35. Thirty-one percent of patients were New Zealand Europeans, 25% Samoan, 17% other Pacific peoples, 15% Maori, 6% Indian, 4% other
European, and 2% Middle-Eastern. None of the patients had past ocular history of elevated intraocular pressure or glaucoma.

Seventy-five percent of subjects did not have any family history of glaucoma, and in 23% this was unknown. Only one patient had a known family history of glaucoma in a first-degree relative and this subject did not have family history of keratoconus. Nineteen percent of eyes had a history of previous topical corticosteroid use; in all cases this had been used in the treatment of an episode of acute hydrops. Seventy-nine percent had not had previous topical corticosteroid use, and in 2% this information was unknown. Twenty-five percent had previous systemic corticosteroid use in the form of asthma inhalers or oral corticosteroids for asthma; 67% had not had previous systemic steroids, and in 8% this was unknown.

The median age of patients undergoing penetrating keratoplasty in this study was 29 years (range 13 to 59). The mean ± SD pre-operative best-corrected Snellen visual acuity was 3/36 (mean logMAR 0.78 ±0.49). Reliable pre-operative intraocular pressure was available for 42 eyes (74%) and the mean was 12.5 ± 2.8 mmHg. Due to severity of disease only 25% of eyes had reliable pre-operative central corneal thickness measurement available on Orbscan II topography despite attempts at recording this on all patients; in these cases the mean central corneal thickness was 391.07 ± 88.12 µm. The first available mean central corneal thickness post-operation between 6 weeks and 12-months was 503.2 ± 80.8 µm; this was unavailable in 18 eyes. Subjects applied topical corticosteroids (of any preparation) for a mean number of 315 ± 65 days (range 118 - 365).
Eighteen eyes (32%) of seventeen patients (35%) had elevated intraocular pressure at some point during the 12-month follow-up period (table 9.1): One at 1-month, nine eyes at 3-months, five at 6-months, one at 9-months and two at 12-months (median 3-months). Of these patients, nine were New Zealand Europeans, two other Europeans, two Indians, one Maori, and three Pacific peoples. The male: female ratio was 12:5. The median age at time of penetrating keratoplasty of this group was 33.5 years (range 18 – 59). Six of these eyes had mildly elevated IOP, 7 moderately elevated, and 5 severely elevated, i.e. 12 (21%) of 57 eyes had moderate to severe elevation of IOP. The highest recorded IOP was 50 mmHg in a 20-year-old Tongan man at the 3-months post-keratoplasty follow-up. The pressure was lowered immediately with topical ocular antihypertensives as well as oral acetazolamide. This enabled the IOP to drop to 13mmHg within 3 hours. However the patient required topical ocular antihypertensives throughout the rest of the post-operative period until topical steroids were stopped. Four other subjects recorded IOP peaks of greater than 30mmHg, being 32, 32, 34 and 34mmHg respectively. Formal perimetry was not performed during the first 12 months post-keratoplasty in those with transiently elevated IOP, however, clinically no optic disc damage was identified.

If only the first operated eye for each patient was considered, the incidence of elevated IOP was 33% (16 eyes of 48 patients). Only two patients with bilateral keratoplasty had elevated IOP post-surgically. The first patient had elevated IOP in both eyes. In this case, the keratoplasties were one-year apart and the first eye developed mildly elevated IOP (23 mmHg) while the second eye suffered severely
elevated IOP (32 mmHg). Elevation of IOP in each eye was first noted at 6-months post-keratoplasty. The second patient experienced raised IOP only in the second eye to undergo corneal transplantation. The operations were 13-months apart for this subject and elevated IOP (28 mmHg) only detected at the 12-months post-surgery clinic review.

Table 9.2 Treatment methods used to treat elevated IOP from 1-week to 12-months post-keratoplasty

<table>
<thead>
<tr>
<th>Treatment methods for elevated IOP</th>
<th>Number of eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduction or cessation of corticosteroids alone</td>
<td>5</td>
</tr>
<tr>
<td>Addition of ocular antihypertensive alone</td>
<td>1</td>
</tr>
<tr>
<td>Addition of ocular antihypertensive initially but reduction of corticosteroids required</td>
<td>4</td>
</tr>
<tr>
<td>Addition of ocular antihypertensive and reduction of corticosteroids simultaneously</td>
<td>7</td>
</tr>
<tr>
<td>Reduction of corticosteroids, addition of ocular antihypertensive and oral acetazolamide simultaneously</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 9.2 outlines the treatment methods used to normalise IOP in those with elevated pressures from 1-week to 12-months post-keratoplasty, including 39% of eyes requiring simultaneous addition of an ocular antihypertensive as well as reduction of topical corticosteroids; 28% requiring reduction or cessation of topical corticosteroids alone; 22% received treatment by addition of topical ocular antihypertensives initially but reduction of corticosteroids was necessary later.
None of the patients required surgical management of elevated IOP. None of the eyes required ongoing treatment with an ocular antihypertensive agent once the IOP normalised and the corticosteroids were withdrawn. None of the eyes demonstrated IOP elevation during the follow-up period once topical corticosteroid was stopped.

Comparing those who experienced elevated IOP to those who did not, the IOP responders were less likely to be of Maori or Pacific ethnicity, and this was statistically significant (p=0.02). However, no statistical difference was found when comparing age (p=0.89), gender (p=0.96), history of previous ocular (p=0.33) or systemic (p=0.82) steroid administration, family history of keratoconus (p=0.40), pre-operative IOP (p=0.53), continuous and interrupted sutures or interrupted sutures only (p=0.26), or post-operative central corneal thickness (p=0.59).

The mean cumulative number of steroid drops at the point of IOP rise for the 18 eyes with elevated IOP was 630.1 (±346.5). The mean 12-month cumulative steroid dose for the IOP responders was 929.8 (±310.3) and for the non IOP-responders was 1228.2 (±338.1) (p=0.003) – due to cessation or reduction of topical corticosteroids in the IOP responder group. None of the eyes experienced allograft rejection as a result of reduction of corticosteroids, and specifically, rejection did not occur more frequently in those whose corticosteroids were reduced due to elevated IOP (p=0.185).
9.5 Discussion

In this study we examined the occurrence of intraocular pressure elevation in patients undergoing penetrating keratoplasty for keratoconus. To the author’s knowledge this is the first study to closely examine elevated intraocular pressure post-keratoplasty in a large cohort of keratoconic subjects selected to exclude other risk factors.

Table 9.3 Comparison with other studies of percentage of post-keratoplasty elevated IOP in keratoconus

<table>
<thead>
<tr>
<th>Author</th>
<th>Country</th>
<th>Number of grafts for keratoconus</th>
<th>Percentage of keratoconic eyes with elevated IOP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goldberg et al. 11</td>
<td>USA</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Kirkness and Moshegov 14</td>
<td>UK</td>
<td>99</td>
<td>12</td>
</tr>
<tr>
<td>Simmons et al. 13</td>
<td>USA</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>Kirkness and Ficker 3</td>
<td>UK</td>
<td>202</td>
<td>2</td>
</tr>
<tr>
<td>Sihota et al. 8</td>
<td>India</td>
<td>26</td>
<td>4</td>
</tr>
<tr>
<td>Fan et al.</td>
<td>New Zealand</td>
<td>57</td>
<td>32</td>
</tr>
</tbody>
</table>
This study confirms our suspicions that the incidence of post-keratoplasty elevated IOP is substantial between 1-week to 12-months post-keratoplasty - at 32%, with 11% of eyes developing mild, 12% moderate, and 9% severe IOP elevation. The incidence of this potentially deleterious effect of topical corticosteroids is significantly higher than that reported previously for keratoconus (see table 9.3). Part of the discrepancy may be due to the differences in the inclusion and exclusion criteria between the studies. For example, in the study conducted by Kirkness and Ficker, steroid responders were excluded from the study as they were examining persistent elevated IOP following keratoplasty; Goldberg et al. defined elevated IOP as being 25 mmHg or higher, whereas our criteria for elevated IOP started at 22 mmHg. However, even excluding those with mildly elevated IOP the current study still demonstrates a higher incidence (21%) for moderate to severe elevation of IOP post-keratoplasty. Potential confounders in earlier studies included the inclusion of significant number of subjects who received combined surgery, or eyes that were aphakic, however, as they did not differentiate these factors by diagnosis of corneal condition, a direct comparison cannot be made with the current study as to whether these variables altered the outcome of IOP in keratoconus.

Other than corticosteroid-induced IOP elevation, several factors have previously been reported to be associated with late elevation in IOP post-keratoplasty; these include: preoperative glaucoma or elevated IOP, aphakia, penetrating keratoplasty combined with anterior segment reconstruction and anterior vitrectomy, and repeat penetrating keratoplasty. As the current study excluded all of these co-morbid conditions as possible confounders and
concentrated on subjects with keratoconus in isolation, the factors most likely to be responsible for elevating the IOP in these subjects are penetrating keratoplasty per se or the IOP response to corticosteroids. However, since all eyes except one required treatment modification by reduction or cessation of steroids (with or without topical ocular antihypertensives) in order to normalise IOP, and subsequently no eyes demonstrated elevated IOP following cessation of corticosteroids, a steroid related elevation of IOP seems the most likely mechanism in this study. With half of the subjects having developed elevated IOP by 3 months postoperatively, the timing of the IOP elevation is also consistent with corticosteroid-induced ocular hypertension, which usually occurs within a few weeks of treatment with potent corticosteroids, or within months with less potent steroids.¹

Corticosteroid-induced ocular hypertension is an incompletely understood phenomenon. Most evidence suggests that the cause of corticosteroid-induced hypertension is a reduction in outflow of aqueous fluid through the trabecular meshwork in the anterior chamber of the eye in susceptible individuals.¹⁵ Risk factors postulated to be associated with steroid response, some shared with those for post-keratoplasty IOP elevation, include a history of glaucoma or glaucoma suspect,¹⁶ older age,¹⁴ and previous steroid response. Whilst 18-36% of the general population have previously been found to have an increase of 5 mmHg or more in IOP after topical corticosteroids,¹⁵,¹⁷ 5-6% of the general population and 46-92% of patients with primary open-angle glaucoma experience significant and potentially damaging rise in IOP after topical steroids administration. Therefore although the overall proportion of steroid responders per se in this study is not
higher than what is reasonable to encounter by chance, the percentage of patients with a moderate or severe IOP rise is much higher.

It is perhaps surprising that previous studies assessing PKP in keratoconus have not demonstrated the same level of steroid responsiveness in their study populations. Kirkness and Moshegov found only 5% of all eyes had raised IOP that responded to manipulation of steroids.\textsuperscript{14} As previously noted, Kirkness and Ficker excluded all steroid responders,\textsuperscript{3} and many other papers have failed to comment on steroid-induced IOP elevation at all.

Comparing patients who developed postoperative IOP elevation to those who did not, the only factor that could be significantly correlated with IOP elevation was ethnicity. The Caucasian subjects of European origin were much more likely to develop postoperative IOP elevation compared with Maori and Pacific peoples in the current study. This is surprising as one might assume that ethnicities unique to New Zealand might account at least in part for the significantly higher incidence of IOP elevation in this study compared to the established studies primarily conducted in Europe and USA.

Little has been published on aqueous humour dynamics in keratoconus; Goodman \textit{et al.} found that there was no difference in mean aqueous flow rates in keratoconus compared to controls, and proposed that the lower mean IOP in this population is due to increased outflow facility.\textsuperscript{19} This could explain the lower rate of IOP elevation post-keratoplasty in keratoconus in other studies, but helps little to explain the current observations. As it is well established that there is a genetic
basis to both keratoconus and primary open-angle glaucoma,\textsuperscript{19,20} one must consider the possibility that the keratoconic population in New Zealand may represent a distinct genotype that exhibits a linkage between the gene(s) responsible for glaucoma and those responsible for development of steroid-induced ocular hypertension.

Although an individual may be considered as either a steroid-responder or not, the cumulative dose of corticosteroids required to “turn-on” the steroid-responsiveness varies from individual to individual and possibly between eyes. This may explain why, in the current series, one patient with identical bilateral penetrating keratoplasty procedures and identical post-operative steroid regimens only experienced elevated IOP in the second eye to undergo surgery. (Interestingly, however, following the 12-month closure date of the current study - at 18-months post-keratoplasty the subject developed significant elevation of IOP in the first eye during an episode of graft rejection and treatment with intensive hourly Prednisolone acetate drops.)

Whilst the mean 12-month cumulative steroid dose was significantly lower for those with elevated IOP than those without IOP elevation, this is due to the fact that a patient was much more likely to have their corticosteroid dose reduced due to IOP elevation than if the IOP remained normal. Although there is no “routine” post-operative topical corticosteroid regime that is widely accepted in the published literature,\textsuperscript{8,12,21} our routine management for all post-corneal transplant cases is gutte prednisone acetate 1% QDS for six months. Whilst it has been well-established that long-term topical steroid treatment improves graft survival
following normal-risk penetrating keratoplasty,\textsuperscript{22} those with reduced corticosteroids in this cohort were not found to be more likely to develop graft rejection during the study period. However, as it has been suggested that steroid responders may be at higher risk of developing glaucoma later in life,\textsuperscript{23,24} glaucoma screening in this population is advisable. Future genetic studies into this group of patients may help confirm our postulation.

In conclusion, this study has revealed a higher incidence of post-keratoplasty IOP elevation in keratoconus subjects than previously reported, yet the populations unique to this study, Maori and Pacific peoples, were associated with a lower likelihood of this complication. Whilst no direct relationship between total dosage of topical corticosteroid and IOP elevation post-keratoplasty was identified, all but one of the 18 eyes that experienced elevated IOP regained normal pressure once the topical corticosteroid was reduced or stopped. Given that elevated IOP may not only affect the success of the corneal transplant but also cause glaucomatous optic neuropathy, further clinical and genetic studies into the links between keratoconus, steroid-induced ocular hypertension and glaucoma might improve the current understanding and optimise clinical management of this condition.
Chapter 10

Measurement of IOP in keratoconus: comparison of Corvis Scheimpflug tonometer with Goldmann applanation, rebound, and dynamic contour tonometry
10.1 Abstract

Purpose
To evaluate the agreement of intraocular pressure (IOP) measurements obtained by the Corvis Scheimpflug tonometer (CST) versus Goldmann applanation tonometry (GAT), ICare rebound tonometer (ICT), and Pascal dynamic contour tonometer (PDCT) in keratoconic corneas.

Methods
A prospective study comparing the IOP obtained by the CST with values obtained by GAT, ICT and PDCT in eyes with, and without, keratoconus. The relationship of the IOP measurements and corneal biomechanical properties were analysed. Pachymetric values obtained by the CST were also compared with ultrasound pachymetry in the same patient population.

Results
The CST demonstrated the least agreement with GAT in eyes with keratoconus with an increase in difference between the devices as IOP increased. Multiple regression analysis demonstrated that CST IOP is strongly correlated with deformation amplitude (p<0.001) and maximum keratometry (P=0.003), whereas GAT IOP did not demonstrate correlation with corneal parameters. In eyes with normal corneas, GAT and CST demonstrated the best agreement in IOP measurement. Poor agreement was observed between the CST pachymetry with ultrasound pachymetry.
Conclusions:

In this pilot study assessing IOP in keratoconus, Corvis ST provided similar values to GAT in normal eyes but Corvis ST reported IOP values were strongly associated with corneal biomechanical factors in eyes with keratoconus. Nonetheless, Corvis ST provided the greatest inter-observer measurement repeatability in keratoconus in the current study. However, Corvis ST currently makes no adjustment for the corneal biomechanical factors which it routinely measures. It cannot be used interchangeably, in abnormal corneas, with the other three tonometers assessed.
10.2 Introduction

It is widely accepted that the ideal tonometer for the measurement of intraocular pressure (IOP) should be accurate, able to provide reproducible results, and be minimally invasive. The Goldmann applanation tonometer (GAT) is currently regarded by many as the gold standard in IOP measurement as it most closely adheres to the stated characteristics of the ideal tonometer. However, it is equally well-recognised that the accuracy of IOP obtained with the GAT is affected by intrinsic corneal properties. Variations in central corneal thickness, corneal curvature, and corneal biomechanical integrity can all influence the accuracy of the GAT. Despite these limitations GAT it is still the most frequently utilised technique in the ophthalmologist’s practice.

Keratoconus is a condition in which accurate IOP measurement poses a significant challenge, since not only is the cornea thinner and the curvature steeper, the biomechanical properties of the cornea are also dramatically altered. Therefore, theoretically if a device can produce accurate measurement of IOP in keratoconus, it may also be useful in corneal disease or post-surgical situations with altered corneal properties.

The Corvis ST (CST, Oculus; Wetzlar, Germany) is a relatively new non-contact tonometer that emits a pulse of air which planaplates and indents the cornea (see figure 10.1). Its proposed advantage over other non-contact tonometers resides in the integral ultra-high-speed Scheimpflug camera. This camera gathers 4330 frames per second of in vivo cross-sectional images in a 100 millisecond period,
thus recording dynamic deformation of the cornea before and after the air puff to calculate IOP value.\textsuperscript{31} Its stated measurement range is from 1 to 60 mmHg. The CST also assesses pachymetry and the biomechanical responses of the cornea to indentation, such as time at first flattening of the cornea (A1), corneal displacement at highest concavity (deformation amplitude, DA), and radius of curvature at highest concavity (RoC).

**Figure 10.1** Corvis Scheimpflug imaging of the cornea at maximal concavity during applanation.

The intra- and inter-observer repeatability of the CST in eyes with normal corneas has been established in earlier studies.\textsuperscript{31,32} It’s accuracy compared to ultrasound pachymetry in the measurement of central corneal thickness in normal corneas
have also been established.\textsuperscript{33} However, being a relatively new device, there are conflicting reports in the published literature regarding the efficacy of the CST to accurately measure IOP.\textsuperscript{31,32,34} Furthermore, the utility of the CST in measuring IOP in corneas beyond the “normal” range has yet to be established. The current study was designed to assess the CST in the keratoconic and normal cornea in relation to: instrument repeatability; measured IOP in comparison to contemporary tonometers; correlation of corneal parameters with IOP measurement; and comparison of central corneal thickness by CST compared to ultrasound pachymetry.

10.3 Methods

Study aims

1) To establish the inter-observer repeatability of the CST in eyes with keratoconus.

2) To compare IOP values as measured by the CST, GAT, ICare\textsuperscript{®} tonometer (ICT) (Tiolat, Oy, Helsinki, Finland), and Pascal dynamic contour tonometer (PDCT) (SMT Swiss Microtechnology AG, Port, Switzerland).

3) To investigate whether corneal parameters including central corneal thickness (CCT), the maximal simulated keratometry value (Kmax), and deformation amplitude correlate to IOP measurement by each tonometer.

4) To compare the central corneal thickness (CCT) values measured by the CST with ultrasound corneal pachymetry (Pachmate DGH 55, DGH Technology, PA, USA).
Patients

Patients with keratoconus (KC) were recruited from anterior segment clinics at Greenlane Clinical Centre, Auckland District Health Board, New Zealand. Subjects with healthy corneas (reference group) were recruited from staff and accompanying persons of patients at the same centre. Exclusion criteria for healthy subjects were: any previous ocular trauma or surgery, contact lens wear, any signs of forme fruste keratoconus based on corneal tomography, or any ocular or systemic disease that may affect the cornea.

Demographic data including age, gender and ethnicity were collected. Only one eye from each subject was examined and was selected at random. In the keratoconic group, eyes with a history of hydrops or forme fruste keratoconus were excluded.

Institutional Review Board (IRB)/Ethics Committee approval was obtained. Informed consent was also obtained from each patient after explanation of the nature and possible consequences of the study prior to participation. This study adhered to the tenets of the Declaration of Helsinki.

Examinations

All examinations were performed by at least one experienced investigator (JCFG and/or NQA). All eyes were examined by slit-lamp biomicroscopy to determine the health status of the cornea.

Computerised tomography was performed using the Pentacam HR (Oculus,
Wetzlar, Germany) to obtain measurements of the corneal thickness and simulated keratometry values prior to any contact investigations.

Immediately prior to IOP measurement, one drop of topical benoxinate hydrochloride 0.4% was administered to the eye to be examined. IOP was measured by four instruments in a random order, using CST, GAT, ICT and PDCT.

Immediately following IOP measurements, CCT measurements were obtained by ultrasound pachymetry using the Pachmate DGH 55.

Corvis ST examination was performed by positioning the patient onto the chin-rest, ensuring centration of the instrument on the cornea using the four red alignment markers on the computer screen. Once centred, the instrument automatically emits a focused puff of air at a pressure of 60 mmHg from a nozzle, which is 3.05 mm in diameter aimed at the cornea from a distance of 11 mm. The Scheimpflug camera is angled at 45° and captures a video sequence of the first inward applanation or flattening of the cornea, followed by deformation at maximal concavity, and finally the second applanation, or second flattening of the cornea as it resumes its original contour.

The Goldman applanation tonometer, ICare® tonometer, and Pascal dynamic contour tonometer were all utilised in accordance with their manufacturer’s recommended measurement technique.
Repeatability
The intra-observer repeatability of Corvis ST has recently been established by the authors using an identical instrument and therefore was not repeated in this study. (Ali NQ et al., in press) The Bland-Altman methodology for measurement error was used to test the inter-observer repeatability of all CST parameters. The mean within-subject standard deviation $S_w$ was calculated. Precision (Pr) was calculated using the formula $1.96 \times S_w$ and repeatability (R) was calculated using the formula $2.77 \times S_w$. The coefficient of variation (CV) was calculated by the dividing the mean of within-subject means by $S_w$. Intra-class correlation (ICC) was calculated using ANOVA. An interclass correlation of more than 0.75, and a co-efficient of variation less than 20% were considered repeatable.

Statistical Analyses
Statistical analysis was performed with the assistance of a biostatistician using Microsoft Office Excel 2003 (Microsoft, Redmond, Washington, USA) and SPSS software version 19.0 (SPSS, IBM, Chicago, Illinois, USA). Statistical $P$ values of 0.05 or less were considered significant.

10.4 Results
Twenty-nine eyes of 29 keratoconic patients and 19 eyes of 19 normal patients were recruited for the study. The average age was 35 ± 13 years in the keratoconic group, and 45 ± 13 years in non-keratoconic subjects. The keratoconic group had more males (59%) than the control group (47%). IOP measurements with each tonometer were normally distributed as confirmed
by the Shapiro-Wilk test (Keratoconics: GAT p=0.17, PDCT p=0.12, ICT p=0.49, CST p=0.87; Controls: GAT p=0.05, PDCT p=0.15, ICT p=0.1, CST p=0.50). In the keratoconus group, IOP measurement by PDCT was obtained to a good quality score (Q-score of 1, 2 or 3) in only 13 eyes (45%), and in only 8 eyes in the normal group (40%). Patient compliance was poorer in this group compared to other techniques utilised in this study.

Twenty patients in the keratoconic group and 19 patients in the normal group underwent examination by two observers.

In the keratoconus group, inter-observer repeatability of IOP measurement by the CST was excellent (ICC=0.9, CV 9.0%, 95% CI 0.78-0.96 p<0.001) compared to ICT (ICC=0.7, CV 16.5%, 95% CI 0.28-0.90 p<0.001), and GAT, which was the least repeatable, although both observers were most experienced with this instrument (ICC=0.47, CV 21%, 95% CI -0.01-0.78, p=0.007).

In the normal group, inter-observer repeatability was similar with the CST (ICC=0.66, CV 6%, 95% CI 0.31-0.86, p=0.002), GAT (ICC=0.59, CV 15%, 95% CI 0.10-0.83 p<0.001) and ICT (ICC=0.62, CV 16%, 95% CI 0.26-0.84 p=0.002). There was less variation in measurements taken by the CST.
Table 10.1  Mean intra-ocular pressure measurements by the Corvis ST compared to Goldmann applanation tonometer, ICare rebound tonometer and Pascal dynamic contour tonometer in normal and keratoconic eyes.

<table>
<thead>
<tr>
<th>Tonometer and Group</th>
<th>Number of eyes</th>
<th>Mean IOP ± SD (mmHg)</th>
<th>Range (mmHg)</th>
<th>Mean difference in IOP compared to GAT (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratoconus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAT</td>
<td>29</td>
<td>11 ±3</td>
<td>2 - 19</td>
<td>1.9 ±2.5 (95% CI 0.9 to 2.9, p&lt;0.001)</td>
</tr>
<tr>
<td>ICT</td>
<td>29</td>
<td>9.6 ±3.3</td>
<td>3 - 16</td>
<td></td>
</tr>
<tr>
<td>PDCT</td>
<td>16*</td>
<td>13.7 ±3.6</td>
<td>6.6 - 18.9</td>
<td>-1.4 ±5.0 (95% CI -4.5 to 1.6, p=0.32)</td>
</tr>
<tr>
<td>Corvis ST</td>
<td>29</td>
<td>10.7 ±3.1</td>
<td>3.5 - 18.5</td>
<td>0.8 ±3.7 (95% CI -0.6 to 2.1, p=0.27)</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAT</td>
<td>19</td>
<td>13 ±3</td>
<td>8 - 20</td>
<td>----</td>
</tr>
<tr>
<td>ICT</td>
<td>19</td>
<td>13.8 ±3.7</td>
<td>9 - 25</td>
<td>-0.2 ±2.5 (95% CI -1.4 to 1.0, p=0.73)</td>
</tr>
<tr>
<td>PDCT</td>
<td>8*</td>
<td>14.9 ±2.4</td>
<td>10.1 - 17.1</td>
<td>-1.4 ±1.7 (95% CI -2.8 to -0.03, p=0.05)</td>
</tr>
<tr>
<td>Corvis ST</td>
<td>19</td>
<td>13.6 ±1.6</td>
<td>11 - 17</td>
<td>0.1 ±2.1 (95% CI -0.9 to 1.1, p=0.83)</td>
</tr>
</tbody>
</table>

GAT=Goldmann applanation tonometer, ICT = ICare rebound tonometer, PDCT= Pascal dynamic contour tonometer
*PDCT measurement was not obtainable or had a quality score of more than 3 in the remainder of patients in the group.

Overall, the CST had the smallest mean difference in IOP measurement compared to the GAT (0.8 ± 3.6 mmHg in keratoconic eyes, and 0.1 ± 2.1 mmHg in normal eyes). (Table 10.1)

In the keratoconus group, the differences in IOP measurements between GAT and ICT, and GAT and CST, were normally distributed, but not between GAT and PDCT (p=0.18, p=0.94 and p=0.04 respectively). In the normal group, the differences in IOP measurements between GAT and ICT, CST and PDCT, and
ICT and CST were normally distributed (p=0.79, p=0.06, p=0.96, p=0.45).

In keratoconic eyes, GAT and ICT showed the best agreement, however ICT gave a higher IOP measurement (+1.9mmHg, limits of agreement -3.1 – 6.9 mmHg) (figure 10.2.1a). The difference in intraocular pressure between GAT and CST increased as the magnitude of IOP increased (figure 10.2.1b). Therefore a log transformation was required to identify any relationship between the two devices (figure 10.2.1c). This revealed that the CST provided the least agreement with GAT, with a 95% confidence interval for the difference in CST IOP being 50% below or 100% above the GAT IOP. That is, at lower intra-ocular pressures, the CST has reasonable agreement with the GAT, whereas at higher intra-ocular pressures there is poor agreement.

Comparison was also made between IOP reading obtained by the CST with that from the ICT, and the CST was revealed to provide overall lower IOP measurements than the ICT (-1.1mmHg, limits of agreement -7.5 – 5.2 mmHg) (figure 10.2.2d). The PDCT and CST also had poor agreement (figure 10.2.2e). In contrast, in normal eyes, CST had the best agreement with GAT (mean difference 0.2mmHg, limits of agreement -4.8 – 5.2 mmHg) (figure 10.3.1a), compared to GAT and ICT (figure 10.3.1b), ICT and CST (figure 10.3.2c) and PDCT and CST (figure 10.3.2d).

Pearson’s correlation testing showed that in keratoconic eyes, IOP measurements made by the GAT and ICT correlated with Kmax, whereas PDCT and CST measurements did not.
\textbf{Figure 10.2.1 and 10.2.2} Bland-Altman plots of the agreement between different tonometers in the measurement of IOP in eyes with keratoconus (plots on following pages).
Figure 10.2.1

a. Bland-Altman plot of the agreement of intraocular pressure measurements by the Goldmann applanation tonometer and the iCare tonometer in keratoconus.

b. Bland-Altman plot of the agreement between Goldmann applanation tonometer and the Corvis ST intracocular pressure measurements in keratoconus.

c. Bland-Altman plot of the agreement of the log of intraocular pressure measurements by the Goldmann applanation tonometer and Corvis ST in keratoconus.
There was no correlation between IOP measurements by GAT, CST, PDCT, or ICT with CCT in keratoconus (table 10.2). There was also no correlation between Kmax or CCT and the difference in IOP measurements between GAT and CST, GAT and ICT, or ICT and CST.
Table 10.2  Pearson's correlation agreement of intraocular pressure measurements in 4 tonometers with the steepest keratometry value (Kmax), central corneal thickness (CCT), and deformation amplitude (DA) in normal and keratoconic eyes.

<table>
<thead>
<tr>
<th>Keratoc-</th>
<th>GAT</th>
<th>ICT</th>
<th>PDCT</th>
<th>CST</th>
<th>Difference GAT-ICT</th>
<th>Difference GAT-CST</th>
<th>Difference ICT-CST</th>
</tr>
</thead>
<tbody>
<tr>
<td>onic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eyes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kmax</td>
<td>0.01*</td>
<td>0.002*</td>
<td>0.59</td>
<td>0.09</td>
<td>0.61</td>
<td>0.43</td>
<td>0.19</td>
</tr>
<tr>
<td>CCT</td>
<td>0.08</td>
<td>0.11</td>
<td>0.98</td>
<td>0.15</td>
<td>0.84</td>
<td>0.74</td>
<td>0.83</td>
</tr>
<tr>
<td>DA</td>
<td>0.006*</td>
<td>&lt;0.001*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.56</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eyes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kmax</td>
<td>0.21</td>
<td>0.84</td>
<td>0.62</td>
<td>0.71</td>
<td>0.35</td>
<td>0.22</td>
<td>0.96</td>
</tr>
<tr>
<td>CCT</td>
<td>0.07</td>
<td>0.04*</td>
<td>0.16</td>
<td>0.67</td>
<td>0.24</td>
<td>0.07</td>
<td>0.02*</td>
</tr>
<tr>
<td>DA</td>
<td>0.02</td>
<td></td>
<td>0.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significance 2-tailed.

CCT = central corneal thickness; CST = Corvis ST; DA = Deformation amplitude; GAT = Goldmann applannation tonometer; ICT = ICare rebound tonometer; Kmax = Maximum simulated keratometry; PDCT = Pascal dynamic contour tonometer.

In eyes with normal corneas, the IOP measurements made by the ICT were correlated to CCT (p=0.04), but not Kmax (p=0.84). However, there was no correlation of IOP measurements by the GAT, CST or PDCT with CCT or Kmax (table 10.2).

The relationship between deformation amplitude (DA) and IOP was used to compare GAT and CST in an attempt to account for the increased difference in IOP between the two devices as the magnitude of IOP increased. Pearson's correlation testing showed significant association between IOP measurements made by both the GAT and CST and DA in eyes with keratoconus (p=0.006 and p<0.001, respectively) (table 10.2).
Multiple regression analysis further confirmed significant associations between the IOP measured by CST and DA, and also revealed an association with Kmax that was not demonstrated on Pearson’s correlation testing. However, on multiple regression analysis, the correlation between GAT and DA and Kmax no longer exist (table 10.3). There is no correlation between the difference in IOP produced by the GAT and CST and DA (p=0.56).

**Table 10.3** Multiple regression analysis assessing the correlation between steepest keratometry (Kmax), central corneal thickness (CCT), deformation amplitude (DA) and IOP measured by Goldmann applanation tonometer (GAT) and Corvis ST (CST) in keratoconic eyes.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Corvis ST Intra Ocular Pressure</th>
<th>Goldmann Intra Ocular Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Co-efficient</td>
<td>P-value</td>
</tr>
<tr>
<td>Deformation Amplitude</td>
<td>-21.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maximum Simulated Keratometry Value</td>
<td>0.3</td>
<td>0.003</td>
</tr>
<tr>
<td>Central Corneal Thickness</td>
<td>-0.002</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Central corneal thickness measurements taken by ultrasound pachymetry were compared to measurements taken by the Corvis ST. The differences in values between the two devices were normally distributed in normal and keratoconic corneas (Shapiro-Wilk Test p=0.53, and p=0.37 respectively). In subjects with keratoconus, central corneal thickness measurements obtained by CST were on average 41 µm greater than those obtained by ultrasound pachymetry (limits of agreement -41 µm to +78 µm) (figure 10.4a). For eyes with normal corneas, CCT measurements were on average 12 µm greater when obtained by CST than compared to ultrasound pachymetry (limits of agreement -20µm to +30µm) (figure 10.4b).
Figure 10.3.1 and 10.3.2  Bland-Altman plots of the agreement between different tonometers in normal eyes.

Figure 10.3.1

a. Bland-Altman plot of the agreement of intraocular pressure measurements by the Goldmann applanation tonometer and the Corvis ST in normal corneas.

b. Bland-Altman plot of the agreement of intraocular pressure measurements by the Goldmann applanation tonometer and the ICare tonometer in normal eyes.
c. Bland-Altman plot of the agreement of intraocular pressure measurements by the Pascal dynamic contour tonometer and the Corvis ST in normal corneas.

\[
\begin{align*}
\text{Difference in intraocular pressure (mmHg)} & \quad \text{Average intra ocular pressure (mmHg)} \\
\hline
-5 & \quad 0 \quad 2 \quad 4 \quad 6 \quad 8 \quad 10 \quad 12 \quad 14 \quad 16 \quad 18 \\
\end{align*}
\]

+2mmHg (mean +1.96SD)
-1mmHg (mean)
-5mmHg (mean -1.96SD)

---

d. Bland-Altman plot of the agreement of intraocular pressure measurements by the ICare tonometer and the Corvis ST in normal corneas.

\[
\begin{align*}
\text{Difference in intraocular pressure (mmHg)} & \quad \text{Average intra ocular pressure (mmHg)} \\
\hline
-8 & \quad 0 \quad 5 \quad 10 \quad 15 \quad 20 \quad 25 \\
\end{align*}
\]

+6mmHg (mean +1.96SD)
0mmHg (mean)
-6mmHg (mean -1.96SD)
Figure 10.4  Bland-Altman plots of the agreement in central corneal thickness measurements between the Corvis ST and ultrasound pachymetry in keratoconic and normal eyes.
10.5 Discussion

Intraocular pressure measurement is a fundamental element of every ophthalmic assessment. Not only is it critical for the detection and diagnosis of glaucoma, but it is currently the only alterable parameter in the glaucoma disease process. Therefore, an instrument that allows more accurate, repeatable, reproducible measurements in eyes with abnormal corneas would be a welcomed addition to the ophthalmologist’s armamentarium.

This study demonstrates the reliable inter-observer repeatability of the Corvis ST in both normal and keratoconic eyes. While the authors have previously assessed and established the intra-observer repeatability of the CST in keratoconus, and others have assessed both intra- and inter- observer reliability in normal corneas, we believe this is the first study to assess the inter-observer repeatability of the instrument in IOP measurement in eyes with keratoconus. Both intra- and inter-observer repeatability are fundamental in the accuracy of a tonometer, and this observation is reassuring.

Interestingly, inter-observer repeatability was overall worse in the reference “normal” group than the keratoconic group. It was the investigator’s observation that subjects with normal corneas were less tolerant of ocular assessments, including non-contact devices such as the CST. We speculate that it is likely that tolerance of ocular assessments was much higher in the subjects with keratoconus as typically more accustomed to ophthalmic assessment and ocular contact, often for the purpose of contact lens fitting.
In a previous study published by our group on assessing the Pascal dynamic contour tonometer in thirty-one eyes post-corneal transplantation, the PDCT was found to be superior to GAT, the TonoPen, and the Ocular Response Analyser in delivering accurate IOP that is independent of corneal factors. In that study, only 14% of subjects had to be excluded due to poor Q-scores. However, in the current study, a much lower compliance was observed when using the PDCT, delivering acceptable Q-scores in only 50% of subjects.

Perhaps unsurprisingly, GAT proved to be the least repeatable technique in keratoconic eyes. This echoes the observations of other studies that have demonstrated the unpredictable and grossly inaccurate IOP measurements obtained by GAT in corneas with irregular astigmatism; this is attributed to the unpredictable area of contact between the cornea and the tonometer tip. This observation once again emphasises the need for an alternative instrument in corneas that deviate significantly from normal.

This study reflects the findings of other investigators in that IOP measured by the CST correlated well with IOP taken by GAT in eyes with normal corneas, although recently Smedowski et al. demonstrated poor IOP agreement between the two devices in eyes with normal corneas. The current study observed that in keratoconic eyes, CST showed the least agreement with GAT, compared with ICT and PDCT, with an overall higher IOP measurement than GAT. Furthermore, the difference increased with a rise in IOP values. This phenomenon was not observed in eyes with normal corneas. These differences may largely reflect the
alteration in corneal biomechanical parameters in eyes with keratoconus.

When assessed further, in keratoconic eyes, IOP obtained by GAT correlated with Kmax in Pearson’s correlation analysis, whereas CST measurements did not. Somewhat unexpectedly, IOP measurement by neither device was influenced by CCT in our keratoconic population. However, there was a strong correlation between IOP obtained by both devices and DA, i.e. the higher the DA, the lower the IOP, and this correlation was more pronounced for CST than GAT. This finding is consistent with a previous study performed by Ali et al.\textsuperscript{35} Interestingly, on multiple regression analysis, the significance between GAT and DA disappears, leaving only a significant correlation between CST and DA, and CST and Kmax.

It is well-recognised that the thinner the cornea, the lower the estimated IOP produced by GAT due to reduced resistance to applanation.\textsuperscript{40-42} It is also understood that the steeper the cornea, the greater the estimated IOP due to the increased force required to applanate the cornea to the same area of contact,\textsuperscript{43,44} as well as the need for a greater force to displace more fluid from under a steeper cornea than under a flat one.\textsuperscript{45} Therefore, we postulate that the opposing effects of steepness and thinness on IOP measurement may essentially compensate for each other, resulting in theoretically “neutral” IOP.

The Ocular Response Analyser (ORA), another instrument like the CST that is based on air-puff applanation technology, provides two IOP values – a Goldmann-correlated IOP (IOPg) and a corneal-compensated IOP (IOPcc), which adjusts for corneal biomechanical factors.\textsuperscript{46} In contrast, the Corvis ST only provides one IOP
measure and our analysis demonstrates that the IOP value is influenced by corneal factors, possibly more so than GAT. Therefore the increase in difference in IOP between GAT and CST with increase in IOP magnitude could be explained by the greater influence of corneal factors at higher IOP on the CST. This is a common phenomenon with many non-GAT tonometers – which at mid-range IOP in normal corneas produce accurate measurement, but have greatly reduced accuracy in extreme ranges of IOP and in the context of abnormal corneas.\textsuperscript{47,48-53}

The fact that the CST does not correct for corneal biomechanical factors has been confirmed by the manufacturer. However, an updated software that will provide corrected IOP values is expected in the near future (Dr S. Reisdorf, PhD, Oculus; Wetzlar, Germany, written communication, January 2014).

The current study suggests that until we can further ascertain its reliability in abnormal corneas such as Keratoconus, the CST should not be used interchangeably with any of the other devices.

One of the advantages of the Corvis ST is its ease of use, being non-contact and therefore producing no infection risk and no patient discomfort. The relatively loud noise associated with the air-puff may startle patients even if informed in advance, however, none of our patients were troubled enough to decline a repeated test. The simultaneous measurement of IOP and corneal parametric values is also useful and allows the operator to mentally adjust the IOP as DA and Kmax values increase.

The pachymetry provided by Corvis ST demonstrated poor overall agreement with
ultrasound pachymetry, especially in eyes with keratoconus. While the poor agreement is consistent with other studies, our observation that ultrasound CCT values were lower than values obtained by an optical device such as the CST is unexpected. The difference could be explained by several factors: 1) the position of the cone could alter the corneal apex from the centre to the inferotemporal cornea, leading to optical misalignment. 2) the distorted corneal shape in the keratoconic eye increases the difficulty of determining the pupil centre, therefore contributing to the misalignment of the ultrasound probe. 3) optical methods of corneal thickness measurements can give falsely elevated readings as measurements can erroneously be taken from the tear film rather than the true corneal surface. Furthermore, in ultrasound pachymetry of the keratoconic cornea, compression of tissue is likely due to increased elasticity of the diseased cornea.

Given that ultrasound pachymetry is the current gold standard in pachymetry measurements, and that the primary aim of this study was not to determine the reliability of the pachymetry feature of the CST, further studies are required in order to validate the accuracy of this function before we can use it with confidence.

The biggest limitation of our study is the relatively small population size, and as such it is not sufficiently powered to detect small differences in IOP values. The operators were also not blinded to the values obtained with the various devices, and although, with the exception of GAT measurements, these were observer independent, this could present a small bias in data collection.
In conclusion, the Corvis ST demonstrated good inter-observer repeatability in IOP measurements. It has excellent correlation with IOP measured by GAT in eyes with normal corneas, and as such would make a good screening tool as it is easy to use and does not carry the risk of infection and does not require topical anaesthesia, due to its noncontact technique. It is a useful multi-tasking device in that it provides IOP as well as corneal parametric calculations. However, its IOP measurements in eyes with keratoconus is strongly influenced by corneal factors and as such cannot be relied upon (without compensatory calculations) for supplying accurate IOP in eyes with abnormal corneas. Larger trials, ideally with manometric comparison would be of benefit in further validating the utility of this novel device in clinical practice.
References from Section III


55. Ucakhan OO, Ozkan M, Kanpolat A. Corneal thickness measurements in normal and keratoconic eyes: Pentacam comprehensive eye scanner


Section IV

Conclusions
Chapter 12

Conclusions: Novel investigations of keratoconus and its assessment in New Zealand
12.1 Introduction

The inter-related series of studies comprising this thesis was developed to investigate two poorly-understood areas associated with keratoconus: the natural history of acute corneal hydrops and the measurement/variation of intraocular pressure (IOP) in the disease process. The aims of the study are outlined as follows:

1. To investigate and identify the risk factors and predictors of acute hydrops in keratoconus
2. To analyse the microstructural changes that occur in the cornea during acute hydrops in keratoconus through in vivo confocal microscopy
3. To assess the ex vivo cellular and microstructural changes that occur in the cornea following acute hydrops in keratoconus through immunohistochemistry
4. To evaluate the corticosteroid-related incidence and risk of intraocular pressure (IOP) elevation following penetrating keratoplasty in the keratoconic population
5. To evaluate the accuracy of new Corvis Scheimpflug tonometer in the setting of abnormal corneal thinning and shape as occurs in keratoconus, compared to Goldmann applanation, rebound, and dynamic contour tonometry

For the purposes of discussion each of the above areas will be reviewed in turn.
12.2 Studies on keratoconus and acute corneal hydrops

(Section II)

In recent years, due to the arrival of advanced anterior segment imaging techniques, a paradigm shift has taken place in the assessment and management of acute hydrops in keratoconus (Further details are discussed in Chapter 4). Despite this, the risk factors and the pathophysiological changes of acute hydrops are still poorly understood.

Section II, therefore aimed to address these areas.

12.2.1 Predictors of acute hydrops in keratoconus (Chapter 5)

The purpose of this study was to identify predictors, risk factors and other associations of acute hydrops in keratoconus in the New Zealand population.

Conclusions:

- The mean age at diagnosis of keratoconus in the hydrops group was 20.3 ± 7.8 years (range 10 - 47 years); hydrops typically developed 4 years after diagnosis (mean age 24.6 ± 8.4 years).
- Subjects with acute hydrops were more likely to be of Pacific, but less likely to be of New Zealand European, ethnicity than control subjects. In comparison, Maori ethnicity was not found to have a significantly positive or negative association with hydrops.
• The pre-hydrops best-corrected visual acuity of affected eyes was poorer than that of the non-hydrops, keratoconic control subjects (p<0.001) at first presentation to our tertiary referral corneal and contact lens service.

• Eye-rubbing was significantly more common in the hydrops group compared to controls (p=0.011). This is independent of a history of contact lens wear, or a history of atopy.

• Subjects with a history of hydrops were less likely to have family members with keratoconus compared to those in the control group (p=0.05).

• Subjects with a history of hydrops were not more likely to undergo penetrating keratoplasty compared to the controls.

12.2.2 Microstructural changes occurring in the cornea during acute hydrops in keratoconus as analysed by in vivo confocal microscopy (Chapter 6)

The advent of in vivo confocal microscopy (IVCM) exemplifies the new age of anterior segment imaging, providing us with the ability to examine detailed microstructural changes in vivo, under more physiological conditions. This chapter identified and monitored the microstructural changes occurring in the cornea during acute hydrops through a prospective study of ten patients.

Conclusions:

• On IVCM, all corneas exhibited intraepithelial bullae that were predominantly located at the level of the superficial epithelium and wing cell layers. In two cases, epithelial bullae were still visible on IVCM at the final
IVCM examination (28 days and 81 days) despite clinical resolution of oedema. For the remaining 8 cases, epithelial bullae had resolved on IVCM at a mean of 53 ± 25 days (range 14-90 days) after presentation.

- Four corneas exhibited hyper-reflective round cells in the epithelium and stroma that were presumed to be inflammatory cells, and the cells persisted until the conclusion of the study in two corneas. In six corneas, inflammatory cells were not visible at any time point in the study.

- In two cases that subsequently developed corneal neovascularisation, elongated branching cells with small cell bodies were noted in the anterior stroma at 6 and 12 weeks respectively. Three months after presentation, both cases also exhibited unusual stromal cells with large speckled cell bodies and elongated branching cell processes. These cells were presumed to be inflammatory cells that may relate to the development stromal neovascularisation.

12.2.3 Ex vivo cellular and microstructural changes in the cornea following acute hydrops in keratoconus (Chapter 7)

In a follow up study to Chapter 6, the cellular and microstructural changes that occur during acute hydrops were further elucidated through immunohistochemical examination. The corneal buttons of five of the ten subjects assessed in Chapter 6 were collected following penetrating keratoplasty and processed for histological and immunofluorescent analysis.

Conclusions:
• Extensive, presumed chronic, inflammatory changes were identified in the post-hydrops corneas. These include:
  o Immunohistochemical markers for macrophages stained positively in the basal epithelium of one cornea.
  o Lymphocytic deposition was identified in the anterior to mid-stroma of three corneas.
  o Professional antigen presenting (Langerhans) cells were demonstrated in the endothelium of three corneas.
  o Marked laminin deposition was observed in four corneas in the anterior to mid stroma, corresponding to the site of healed hydrops.
• In addition, langerin-positive dendritic cells were identified in the basal epithelium and anterior stroma of two corneas. These cells are likely part of the resident dendritic cell population of the cornea and were not recruited as part of the hydrops process.

12.2.4 Keratoconus and keratoconus surgery-related case studies: Long-term microstructural changes following epikeratophakia and an extreme Descemet's tear in acute hydrops (Chapter 8)

A. Epikeratophakia is a surgical procedure in which a stromal lenticule of donor human corneal tissue is sutured onto the anterior surface of the recipient cornea denuded of epithelium in order to change its anterior curvature and refractive properties. It is most commonly performed for the treatment of keratoconus when other treatment options are not available or appropriate. Though not routinely performed in the developed world, this study of the microstructural changes to the
cornea as analysed by in vivo confocal microscopy still provides insight to the potential adaptations of the cornea at a cellular level.

Conclusions:

- Reduced keratocyte density in both the grafted lenticule and the host stroma were observed.
- The sub-basal nerve plexus was present in the lenticule, although with a reduced nerve density.
- The host endothelium exhibited guttae, enlarged and irregularly-shaped cells similar to that observed in Fuch’s endothelial dystrophy.

B. A case of an extreme Descemet’s tear that resulted from acute hydrops in keratoconus was presented in this chapter. The large Descemet’s tear persisted even following clinical resolution of corneal oedema. The corneal button was retrieved following penetrating keratoplasty and analysed through histology and immunohistochemistry.

Conclusions:

- The edges of the persistent Descemet’s tear, staining positively for laminin, were retracted and curled anteriorly, creating a scroll at each end.
- The clinical and histological resolution of corneal oedema represents the ability of the endothelium to function without and intact Descemet’s membrane.
12.3 Studies of intraocular pressure in keratoconus

(Section III)

Intraocular pressure (IOP) measurement in the altered cornea, such as in keratoconus or post-keratoplasty, is both difficult to assess and unreliable. In keratoconus, not only is the cornea thinner and steeper, the biomechanical parameters are also altered, making conventional methods of IOP measurement erroneously. Following penetrating keratoplasty, there is a further alteration to the biomechanical properties. Furthermore, intensive corticosteroid treatment is often required to reduce inflammation and prevent rejection. Corticosteroids are recognised to alter IOP and this corticosteroid response can be more pronounced in the keratoconic eye. This section aimed to address these issues.

12.3.1 Corticosteroid-related intraocular pressure elevation following penetrating keratoplasty in the keratoconic population (Chapter 9)

The keratoconic cornea poses significant limitations on the accurate measurement of IOP. This is further complicated by the presence of a corneal graft in many keratoconic eyes - not only due to structural changes to corneal shape and thickness, but due to the necessity of prolonged topical corticosteroids to minimise rejection. Anecdotally, keratoconic eyes were reported to have an increased incidence of steroid-related elevation of IOP. Therefore the incidence of corticosteroid-related IOP elevation over a twelve-month follow-up period and potential associations were identified in this study.
Conclusions:

- Thirty-five percent of subjects developed elevated IOP at some point during the 12-month period. This is considerably higher than previously published figures.
- In 78% of eyes, the IOP elevation occurred between 3 and 6 months post-keratoplasty.
- Twenty-one percent of eyes developed moderately to severely elevated IOP (26 mmHg or higher).
- The steroid-responders were less likely to be of Maori or Pacific ethnicity than Caucasian (p=0.02).
- Twenty-eight percent of affected eyes required reduction or cessation of topical corticosteroids alone; 22% received treatment by addition of topical ocular anti-hypertensives initially but reduction of corticosteroids was necessary later; and 39% of eyes required simultaneous addition of an ocular antihypertensive as well as reduction/cessation of topical corticosteroids.
- None of the eyes required surgical intervention to control IOP. None of the eyes required ongoing topical ocular hypotensives once corticosteroids were withdrawn.
- In summary, intraocular pressure elevation in eyes with keratoconus that have undergone corneal transplantation is more common than previously reported but typically responds to reduction or withdrawal of topical steroids and short term treatment with topical ocular anti-hypertensives.
12.3.2 Comparison of Corvis Scheimpflug Tonometer with Goldman applanation, rebound, and dynamic contour tonometry in the measurement of IOP in keratoconus (Chapter 10)

There is no currently available tonometer that is considered to be entirely reliable in the measurement of IOP in the altered/diseased cornea. The Corvis ST (CST, Oculus; Wetzlar, Germany) is a relatively new non-contact tonometer that also has the function of assessing corneal biomechanical factors. It has been reported to be reproducible and accurate in its measurement of IOP in normal corneas. This study aimed to assess the accuracy of the CST in the measurement of IOP in keratoconus, in comparison to Goldmann applanation, rebound, and dynamic contour tonometry.

Conclusions:

- The Corvis Scheimpflug tonometer demonstrated good inter-observer repeatability.
- Although the CST had the least difference in mean IOP compared to the Goldmann applanation tonometer (GAT), it had the least overall agreement to the GAT in keratoconic eyes. This poor agreement was more pronounced at higher IOPs than lower IOPs.
- Multiple regression analysis demonstrated significant association between IOP obtained by the CST with corneal steepness and deformation amplitude in keratoconic eyes. This was not demonstrated with other tonometers.
12.4 Conclusions

Keratoconus is a fascinating, presumed multifactorial, relatively common corneal condition - particularly prevalent in the New Zealand population. Since the first comprehensive description of the disease in 1854, the pursuit of our better understanding of the condition has not ceased – yet many aspects of the disease remain enigmatic.

I believe this series of inter-related clinical and laboratory studies has increased our understanding of the predictors of acute hydrops in keratoconus, revealed both the *in vivo* and *ex vivo* microstructural changes that occur during acute hydrops, identified the incidence of corticosteroid-related IOP elevation in keratoconus and possible risk factors, and assessed the accuracy of a new tonometer in the measurement of IOP in the keratoconic eye.

Several challenges still remain. While the inflammatory changes that occur in acute hydrops have been further established through this research, not all the cellular structures identified have been fully characterised. The potential role of such inflammatory cells in the induction of neovascularisation and scar formation also requires further investigation. While we have identified a racial association to steroid-response in IOP elevation, the genetic basis to steroid-response in keratoconus still remains elusive. Lastly, there is the ongoing pursuit of the perfect tonometer for the assessment of IOP in the diseased cornea in conditions such as keratoconus.
The studies that comprise this doctoral thesis, and their evolution through more than five years of research, have taken me on a longer journey of exploration than initially predicted. However, like all journeys, although many discoveries have been made along the way, many new paths have also been highlighted. These new paths will be fully explored by the author and fellow travelers in the future.
Section V

Appendices
Appendix 1  Papers published from this thesis


Appendix 2  International conference presentations from this thesis

Paper


Poster


